

Advanced Drug Delivery Systems of Curcumin for Cancer Chemoprevention

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Abstract

Since ancient times, chemopreventive agents have been used to treat/prevent several diseases including cancer. They are found to elicit a spectrum of potent responses including anti-inflammatory, antioxidant, antiproliferative, anticarcinogenic, and antiangiogenic activity in various cell cultures and some animal studies. Research over the past 4 decades has shown that chemopreventives affect a number of proteins involved in various molecular pathways that regulate inflammatory and carcinogenic responses in a cell. Various enzymes, transcription factors, receptors, and adhesion proteins are also affected by chemopreventives. Although, these natural compounds have shown significant efficacy in cell culture studies, they elicited limited efficacy in various clinical studies. Their introduction into the clinical setting is hindered largely by their poor solubility, rapid metabolism, or a combination of both, ultimately resulting in poor bioavailability upon oral administration. Therefore, to circumvent these limitations and to ease their transition to clinics, alternate strategies should be explored. Drug delivery systems such as nanoparticles, liposomes, microemulsions, and polymeric implantable devices are emerging as one of the viable alternatives that have been shown to deliver therapeutic concentrations of various potent chemopreventives such as curcumin, ellagic acid, green tea polyphenols, and resveratrol into the systemic circulation. In this review article, we have attempted to provide a comprehensive outlook for these delivery approaches, using curcumin as a model agent, and discussed future strategies to enable the introduction of these highly potent chemopreventives into a physician's armamentarium. *Cancer Prev Res*; 4(8); 1158–71. ©2011 AACR.

Introduction

Currently, clinical and basic research is driven by the aim of curing advanced diseases. This aim is particularly difficult for cancer because of the genetic heterogeneity of the cell types involved. A cancerous lesion usually consists of a family of genetically/phenotypically different cell types that originate over a period of years and when this lesion becomes invasive, cell heterogeneity ultimately complicates the treatment of the disease (1). Therefore, attempts have been made to blunt, if not reverse, this carcinogenic cascade by intervention with agents; an approach coined over 30 years ago by Sporn as chemoprevention (2). Because a typical carcinogenesis process involves many environmental, dietary, occupational, and epigenetic factors that determine its long latent period, intervention with

phytochemicals that have little or no toxicity can provide an alternative strategy for controlling the initiation and progression of this disease. As a result, studies initiated by the National Cancer Institute have led to the screening and identification of thousands of such compounds, of which a few dozen have shown significant preventive/therapeutic potential (1). Although, most of these natural compounds such as curcumin, resveratrol, epigallocatechin gallate (EGCG), and indole-3-carbinol, exhibited potent chemopreventive/antitumor activity in cell culture as well as in some animal studies, they elicited only low activity in various clinical studies (reviewed in ref. 3).

The limited efficacy of several chemopreventives in pre-clinical and clinical studies is attributed largely to their low bioavailability, which results in subtherapeutic concentrations at the target site. To overcome the bioavailability issues, advanced drug delivery systems, designed to provide localized or targeted delivery of these agents, may represent a more viable therapeutic option. Various drug delivery systems such as nanoparticles (NP; refs. 4, 5), liposomes (6, 7), microparticles (8, 9), and implants (10) have been shown to significantly enhance the preventive/therapeutic efficacy of many chemopreventives by increasing their bioavailability and targetability. Therefore, we selected curcumin as a model compound to acquaint the readers with various advanced drug delivery strategies that can be

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successfully used for natural compounds and to help explore other untouched avenues in chemoprevention and therapeutic prevention (inhibition of progression of a disease to advanced malignancies; ref. 11). Although, several drug delivery systems have been developed to harness various activities of curcumin and other natural compounds, we have restricted our discussion mainly to NP, microparticles, liposomes, microemulsions, and polymeric implants because of the relatively detailed understanding of these delivery systems and for their potential to enter into various preclinical and clinical studies.

Curcumin: A Potent Chemopreventive

Although a number of potent chemopreventives have been identified from plant sources, curcumin [a principal bioactive component of *Curcuma longa* (turmeric)], represents one of the most investigated phytochemicals with over 3,770 hits on using curcumin as the search string on Pubmed with about 1,200 hits in the last 2 years alone. There are 3 major curcuminoids that constitute curcumin: curcumin (curcumin I, 75%), demethoxycurcumin (curcumin II, 20%), and bisdemethoxycurcumin (curcumin III, 5%; ref. 12 and our own analysis; Fig. 1). Research over the last 2 decades has shown curcumin to be a potent antioxidant, anti-inflammatory, antiproliferative, antimetastatic, antiangiogenic, antidiabetic, hepatoprotective, antiatherosclerotic, antithrombotic, and antiarthritic agent in cell culture and animal studies (13). Various cell culture studies have shown that it induces apoptosis in oncogenic cells by inhibiting various intracellular transcription factors and secondary messengers such as NF- κ B, AP-1, c-Jun, the JAK-STAT pathway, and various others (13–15). It exhibits potent anti-inflammatory activity, due to the inhibition of κ B kinase required for the activation of NF- κ B, an important transcriptional regulator of inflammatory pathways involved in carcinogenesis and various other pathologic conditions (16–18). Curcumin is well known for its

potential to inhibit carcinogenesis induced by chemical carcinogens, at both initiation and progression stages in various preclinical studies (19). It is known to inhibit cytochrome P450 (CYP) enzyme-mediated bioactivation of environmental carcinogens like benzo[*a*]pyrene (B[*a*]P; ref. 20). As a metabolic substrate and an inducer of CYP1A1, curcumin is postulated to act as a competitive inhibitor of B[*a*]P metabolism, blunting its bioactivation via CYP1A1 (20). Curcumin also increases the levels of other endogenous antioxidants via the Nrf2 pathway to strengthen body's defenses against reactive oxygen species (ROS; ref. 21).

Despite these advantages, curcumin possesses poor water solubility; as a consequence, it exhibits solubility limited bioavailability, which makes it a class II drug in the Biopharmaceutics Classification System (22). Furthermore, due to its rapid intestinal and hepatic metabolism, approximately 60% to 70% of an oral dose of curcumin gets eliminated in the feces (23). In rats, curcumin administered as an aqueous suspension (2 g/kg) provided a maximum plasma concentration of 1 μ g/mL within 1 hour, and dropped rapidly to undetectable levels within 5 hours (24). Studies by Pan and colleagues showed that after intraperitoneal administration of 0.1 g/kg curcumin to mice, only about 2.25 μ g/mL reaches the plasma within 15 minutes which rapidly drops down to 0.35 μ g/mL after 1 hour (23) (Table 1). When curcumin was administered by parenteral routes like intravenous, about 50% was found to be eliminated in bile within 5 hours (25). Even in clinical studies, high doses of orally administered curcumin (8–12 g daily) resulted in very low curcumin concentrations in the plasma (<1 μ g/mL), levels that were not high enough to exert any significant pharmacologic or therapeutic activity (26). In various clinical studies, only a few patients responded to curcumin despite high doses used (Table 2). A very recent clinical study by Carroll and colleagues (27) reported significant efficacy of curcumin in reducing colorectal aberrant crypt foci at 4 g/d dose where 2-gram dose was ineffective. No curcumin was detected in the plasma or biopsy samples from patients when analyzed by UFLC-UV (ultraflow liquid chromatography), although significant levels of curcumin conjugates were found. The authors implicated the efficacy may be due to the sulfate and glucuronide conjugates of curcumin, the notion which will require support from preclinical studies. If the samples were collected several hours after curcumin intake, it is likely that (free) curcumin was available to the target site at least for initial hours to elicit the efficacy. It is, therefore, quite probable that by the time biopsy tissue and plasma were collected, most of the curcumin might have metabolized giving only the glucuronide and sulfate conjugates in the plasma. It has been found that curcumin undergoes rapid metabolism in the intestine (28) and liver to form various active and inactive metabolic products that are further converted into excretable glucuronide and sulfate conjugates. Curcumin provides easily accessible -OH and -OCH₃ sites (Fig. 1) to form conjugates with glucuronides and sulfates that can be deconjugated by glucuronidases and

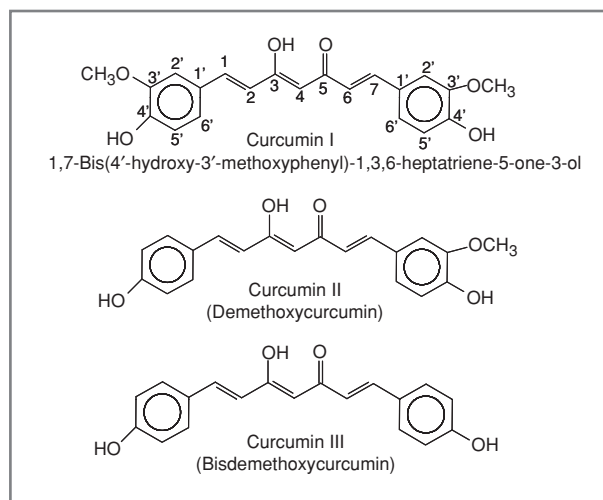


Figure 1. Structures of the 3 curcuminoids as components of curcumin.

Table 1. Examples of some preclinical studies reported with curcumin

Animal	Route	Dose	Findings	Refs.
Rats	Oral	1 g/kg	<ul style="list-style-type: none"> Poorly absorbed 75% excreted in feces 	(40)
Rats	Oral	2% Diet	<ul style="list-style-type: none"> 12 nmol/L in plasma 	(41)
Mice	Intraperitoneal	100 mg/kg	<ul style="list-style-type: none"> 2.25 µg/mL in 15 minutes Disappeared within 3 hours 	(23)
Rats	Intravenous	40 mg/kg	<ul style="list-style-type: none"> Disappeared within 1 hour 	(42)

sulfatases, respectively (29). It has been suggested that this biotransformation of curcumin either occurs in intestine during absorption (30) or in liver (31) coupled with enterohepatic recirculation (32). Studies by Shoba and colleagues (24) showed that coadministration of piperine (20 mg/kg), a potent inhibitor of glucuronidation in the liver and gastrointestinal tract, significantly increases the curcumin bioavailability by 20-fold in humans. These limitations of low solubility, rapid metabolism, and hence low bioavailability have limited the therapeutic success of curcumin in cell culture systems and elicited only limited success in various animal and clinical studies. In last 2 decades, several novel drug delivery systems such as micelles (33), liposomal vesicles (34), NPs (35–37), nanoemulsions (38), phospholipid complexes (39) and polymeric implants (10) have been designed to enhance the bioavailability of curcumin and to enable use of this compound for therapeutic prevention or risk reduction at the precancer stage, which are discussed below.

Nanoparticles

Recently, the development of new drug delivery systems for lipophilic compounds has made tremendous improvements toward enhancing their bioavailability. The advent of nanotechnology has been exploited for the development of various nanoparticulate drug delivery systems that can enable formulation and delivery of curcumin (Table 3) and other hydrophobic drugs which earlier was a conundrum for the formulation scientists (47). These delivery systems

have gained immense popularity in the last decade due to their potential to improve the therapeutic index of the encapsulated drugs either by protecting them from enzymatic degradation (48), by altering their pharmacokinetics (49), by blunting their toxicity (50), or by providing controlled release over extended periods of time (51). According to the National Nanotechnology Initiative (NNI), nanoparticulate delivery systems contain encapsulated, dispersed, adsorbed, or conjugated drugs within a particle size range of 1 to 100 nm (52).

Upon oral administration, colloidal particles like nano/microparticles are absorbed in their intact form via lymphoepithelial M cells of Peyer's patches. These particles that bind to the apical side (toward the lumen) of M cells get internalized and are subsequently shuttled to lymphocytes (53). These colloidal carriers transit slowly in the gut which increases the local concentration gradient across the absorptive segments of the intestine, further enhancing the absorption rate (54). NPs as drug delivery vehicles also enable passive targeting in tumors and other inflamed tissues due to increased vascular leakiness that results because of increased production of cytokines and angiogenesis cascades at these sites. In majority of solid tumors, the vascular cutoff pore size ranges between 380 and 780 nm (55, 56), whereas normal vasculature is impermeable to particles larger than 2 to 4 nm (57, 58). Such a vast difference in vascular permeability enables the passive targeting of tumors and inflamed tissues by NPs and leads to their accumulation resulting in an enhanced permeation

Table 2. Examples of some clinical studies reported with curcumin

Route	Dose	Findings	Refs.
Oral (n = 34)	1–4 g/d for 6 months	<ul style="list-style-type: none"> No reduction in peripheral biomarkers of inflammation. No improvement in cognitive performance in Alzheimer's patients. 	(43)
Oral (n = 21)	8 g/d until disease progression	<ul style="list-style-type: none"> 1 patient showed stable disease (<18 months) and one showed tumor regression with an increase in serum cytokines 22–41 ng/mL peak plasma levels. 	(44)
Oral (n = 25)	8 g/d for 3 months	<ul style="list-style-type: none"> ~1.77 µmol/L plasma concentration peaked at 1–2 hours and declined within 12 hours. 	(45)
Oral (n = 12)	450–3,600 mg/d for 1 week prior to surgery	<ul style="list-style-type: none"> Poorly available, insufficient hepatic levels for inhibition of hepatic metastasis from colorectal cancer. 	(46)

Abbreviation: n, number of patients in the trial.

Table 3. Polymeric and solid lipid NP formulations of curcumin

Formulation	Method	Particle size, nm	Encapsulation efficiency, %	Advantages	Disadvantages	Refs.
PLGA NPs of curcumin for oral administration.	Solvent evaporation diffusion	120-240 (PDI = 0.31)	77	<ul style="list-style-type: none"> • 26-fold increased oral bioavailability as compared with oral curcumin suspension. • Increased biological half-life of curcumin. 		(37)
PLGA and PEG NPs of curcumin for parenteral administration.	Nano-precipitation	80-90	97.5	<ul style="list-style-type: none"> • Enhanced inhibition of TNF-induced NF-κB activation as compared with free curcumin. • Direct systemic administration. 		(35)
NIPAAAM NPs of curcumin containing PEG monoacrylate.	Micellar aggregation	~50	>90	<ul style="list-style-type: none"> • Increased efficacy at lower doses. • 3.3-fold increased residence time on gastric mucosa. 	Thiolation leads to increased particle size.	(51)
PLGA NPs of curcumin coated with thiolated chitosan.	Emulsion solvent evaporation	578 \pm 67 (pH 7.4)	28	<ul style="list-style-type: none"> • Highly porous structure. 		(59)
Butyrylcyanoacrylate NPs of curcumin coated with poloxamer 188.	Anionic polymerization, solvent evaporation	160-240 (PDI ~ 0.25)	78	<ul style="list-style-type: none"> • Higher drug release at acidic pH for intracellular delivery to lysosomes. 	Probability of encapsulation of multiple particles inside PLGA layers.	(65)
NIPAAAM NPs of curcumin multi layered with PLGA.	Free-radical polymerization, double emulsion solvent evaporation	500-1,000	49.5	<ul style="list-style-type: none"> • Potential to deliver both hydrophilic as well as hydrophobic drugs simultaneously. • Increased uptake by macrophages for maximal anti-inflammatory activity. 	Cannot be stored for longer periods of time.	(74)
Surface modified DMPC SLNs for parenteral administration.	Extrusion through 0.2 μ m filter	187 \pm 53	97			

and retention (EPR) effect (55). As a consequence of this passive accumulation at target sites, the concentration of the drug at healthy tissues is correspondingly lower, thereby blunting the intensity of side effects. However, it is noted that for patients with other pathophysiologic conditions that are associated with leaky vasculatures, drug delivery by NPs could result in distribution to multiple sites, thereby blunting (to some extent) selectivity for tumor tissues. Such delivery systems are particularly effective in testing and developing new chemical entities including natural compounds like curcumin that possess suboptimal physicochemical and pharmacokinetic properties to be developed as new drug candidates.

Being lipophilic, curcumin partitions/encapsulates into the hydrophobic core of amphiphilic polymers or phospholipids of NPs which not only enhance its bioavailability but also increase its stability by protecting them from the influence of outside environment (51). One of the most investigated method for preparing such NP formulations is emulsion diffusion evaporation method which involves solubilizing the drug and/or polymer [poly(lactic-co-glycolic acid); PLGA] in any organic solvent like ethyl acetate, followed by its drop wise addition into an aqueous phase that contains a suitable stabilizer to result into an emulsion. The emulsion can then be homogenized and diluted with a large quantity of water so that solvent diffusion can result in nanoprecipitation. This method provides uniformly sized (120–240 nm) spherical NPs of curcumin (Fig. 2), and as solubility of the incorporated drug plays a pivotal role in determining encapsulation efficiency, stabilizers with lower drug solubility were found to be better candidates for achieving high drug encapsulation (37). *In vivo* studies in rats showed that curcumin NPs increased curcumin bioavailability by 26-fold as compared with oral curcumin suspension and by 9-fold as compared with a curcumin suspension administered in conjunction with piperine (37). Furthermore, similar PLGA NPs, prepared by Anand and colleagues (35) using F-68 as the solubilizer, were found to possess similar efficacy as free curcumin in killing tumor cells but a higher potency in inhibiting NF- κ B activation in cell culture, compared with free curcumin. The authors of this study also claimed superior bioavailability from curcumin NPs; this claim, however, is difficult to assess, as curcumin was administered to the mice via intravenous route where bioavailability does not come into play. Nonetheless, an increased half-life of the curcumin in plasma was evident (35). Curcumin NPs can also be prepared using other copolymers like *N*-isopropylacrylamide (NIPAAM), *N*-vinyl-2-pyrrolidone (VP), and polyethylene glycol monoacrylate [NIPAAM (VP/PEG A); ref. 36]. These NPs possess very low polydispersity with an average particle size of 50 nm that enables them to freely permeate into different pancreatic cancer cell lines. Although, these curcumin NPs were found to be equally efficacious as free curcumin in cell culture but had an added advantage of their direct injectability into the systemic circulation, thereby bypassing the oral route (36).

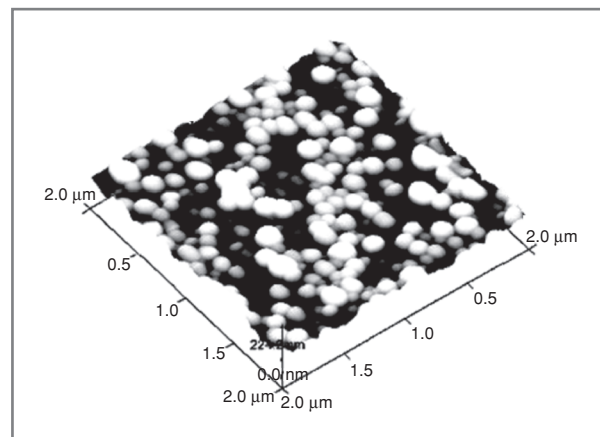


Figure 2. Atomic force microscopy image of curcumin-loaded PLGA NPs prepared by emulsion diffusion evaporation method [Reprinted with permission from Shaikh and colleagues (37)].

Another method to prepare curcumin NPs is by anionic polymerization solvent evaporation method (59). This method involves drop wise addition of a butylcyanoacrylate monomer solution into a constantly stirred acidic ethanol solution containing a suitable surfactant and sodium sulfate. At the critical micelle concentration (CMC), surfactant molecules aggregate together to form a swollen micellar structure containing multiple monomer units. Polymerization of monomer units occurs inside these micelles, forming primary polymer particles that grow in size to form NPs. Curcumin or any other chemopreventive can be added during or after the addition of monomer solution to achieve efficient encapsulation during the growth phase. This method provides uniform NPs [polydispersity index (PDI) = 0.23–0.27] of 160 to 240 nm, with particle size directly related to monomer concentration and inversely related to surfactant concentration (59). Furthermore, it also results in the formation of a highly porous structure with a very high surface area that can be loaded with hydrophobic drugs like curcumin (60). These NPs were found to provide higher drug release under *in vitro* conditions at acidic pH compared with physiologic pH, showing their ability to efficiently deliver their cargo inside the cells after degradation by lysosomes, where conditions are more acidic (59).

The other advantage of using polymeric NPs is their amenability to alterations of surface properties. Different functional groups like thiols can be covalently or noncovalently conjugated with the polymeric chains to increase or decrease the mean residence time of the NPs in the gastrointestinal mucosa. Grabovac and Bernkop-Schnurch prepared such PLGA NPs modified at the surface with thiolated chitosan (51). Thiolated chitosans owing to their -SH groups interact with mucus to form disulfide linkages conferring them with highly mucoadhesive properties and hence an increased residence time (61). Furthermore, due to various inter- and intramolecular disulfide bonds

between chitosan molecules, a tight 3-dimensional structure results providing a controlled release (61). Other mechanisms like reversible opening of tight junctions and inhibition of efflux P-glycoprotein (P-gp) pumps have also been shown to be associated with these thiolated chitosans (62). Although thiolation increases the mean residence time of the coated NPs on the mucosa, it also increases the particle size with decreased encapsulation efficiency of drugs as compared with unmodified NPs (51). The size of curcumin NPs was found to increase from 284–420 nm to 817–960 nm on chitosan coating with half the entrapment efficiency limiting the drug-loading capacity of the thiolated NPs (51).

Another variant of modified NPs is formulation of multi-layered polyionic/polymeric shells encapsulating NPs containing drugs. These polyelectrolyte shells are formed as layers over the surfaces of NPs to alter their cell uptake, to attach tumor targeting agents, to increase stability, and/or to control their loading/release characteristics (63). These layered NPs have been shown using gelatin as the polymer and can be prepared by a 2-step desolvation method followed by formation of layered polyionic shells (64). First, the gelatin NPs are prepared by precipitating gelatin from an acidified solution by slowly adding acetone and then crosslinking gelatin with glutaraldehyde. Then, an aqueous solution of these NPs can be coated with polyionic shells by the sequential addition of polyanions (polystyrene sulfonate, poly-L-glutamic acid, or dextran sulfate) and polycations (polyallylamine HCl, poly-L-lysine, or protamine sulfate) at pH 6. Because gelatin is positively charged at acidic pH, a polyanionic layer forms first followed by a polycationic layer. Once prepared, these NPs can be further added to curcumin solution to adsorb curcumin at their surface via hydrophobic interactions that develop between phenol groups of curcumin and amino acids of gelatin like proline (63).

Such multilayered NPs can also be modified for the targeted delivery of chemopreventives. In such nanostructures, polymeric layers with the entrapped chemopreventives encapsulate a magnetic iron core that acts as a targeting system (65). Efficacy of such multilayered NPs of curcumin was shown by Koppolu and colleagues (65) using poly(NIPAAm) and PLGA as polymers. In this approach, NIPAAm undergoes free-radical polymerization onto the magnetic core via covalent coupling with a silane reagent. The resultant NPs can then be coated with PLGA using a double emulsion solvent evaporation method, yielding NPs of 500 to 1,000 nm in size that can be used to deliver both hydrophilic and hydrophobic chemopreventive compounds simultaneously. The hydrophilic compounds can be loaded into the poly(NIPAAm) layer and the hydrophobic drugs can be loaded into outer PLGA layer (65). However, attachment of multiple poly(NIPAAm) particles at the surface of PLGA particles as well as encapsulation of multiple poly(NIPAAm) particles in the PLGA layer (as opposed to the encapsulation of a single particle) raises some concerns about control and the success of the formulation method.

Targeted delivery of chemopreventives can also be achieved by conjugation of NPs or drugs with ligands like folic acid that can recognize some specific surface attributes of target cell types. Different cancer types often overexpress some specific epitopes or receptors (66) and bioconjugation of chemopreventives to ligands having high specificity for these unique surface receptors can help in achieving their targeted delivery to any cancer type. Salmaso and colleagues (67) showed targeted delivery of curcumin by attaching folic acid (as a ligand) to the polymeric carrier. Presence of folic acid enabled these NPs to undergo clathrin-independent endocytosis into cells that specifically overexpress folic acid receptors. This formulation involved conjugation of PEG, covalently linked to folic acid on one end with isocyanate group of hexamethylene which is further linked to a cyclodextrin curcumin complex on the other end. Hexamethylene is used as a linker to decrease the steric hindrance of bulky PEG chains with cyclodextrin curcumin complex where cyclodextrin was used to bind curcumin into its cavity and to enhance its solubility. These conjugated complexes of curcumin were found to be (i) 3,200 times more soluble, (ii) ~12 times more stable, (iii) 2 times more specific, and (iv) 45 times less degradable at pH 7.2 (the degradation rate constant decreased from 321×10^{-4} to $7 \times 10^{-4} \text{ min}^{-1}$) compared with curcumin alone (67). However, an insufficient cell uptake led to limited beneficial effects of this bioconjugate and further biological investigations are required to show efficient drug release from the conjugates into the tumor cells.

Solid lipid nanoparticles

Solid lipid nanoparticles (SLN) have also shown significant potential for the delivery of lipophilic compounds like curcumin (68). SLNs were first introduced in mid 1990s as novel drug delivery systems (69) capable of protecting the labile drugs from light/pH/heat-mediated degradation, controlled release, and excellent biocompatibility/tolerability (70). These are spherical lipid NPs with a high specific surface area that can be easily modified to (i) attain a favorable zeta potential, (ii) pseudo zero-order kinetics, (iii) rapid internalization by cancer cells, and (iv) impart stealth properties to lessen uptake by the reticuloendothelial system (RES). These properties make them highly versatile drug delivery systems for a variety of compounds with different physicochemical and pharmacologic properties (68). Their lipophilic character enables them to cross the blood–brain barrier (BBB), providing a viable alternative vehicle for the delivery of less lipophilic drugs that cannot cross the BBB (47). Furthermore, biological origin of lipid component of these SLNs renders them less toxic as compared with polymeric NPs (71). This drug delivery carrier not only protects the entrapped drug from photochemical or pH-mediated degradation but also enables drug targeting and easy large-scale production (72, 73). Such characteristics make SLNs as suitable drug delivery carriers for curcumin and other chemopreventives like resveratrol, and β -carotene which owing to their lipid solubility gets localized in the bilayer membrane of lipid

vesicles/NPs and results in enhanced bioavailability. Initially, hot homogenization and warm microemulsion techniques were used for the preparation of SLNs but later other advanced techniques like high-pressure homogenization, solvent emulsification evaporation/diffusion, high-speed stirring, double emulsion method, and ultrasonication were introduced (47).

Curcumin SLNs can be formulated using dimyristoyl phosphatidylcholine (DMPC) via extrusion through a 0.2- μm filter (74). These vesicles were surface modified by L-glutamic acid, *N*-(3-carboxy-1-oxopropyl)-1, 5-dihexadecyl ester, and PEG to increase their uptake by macrophages. Because macrophages produce ROS that leads to oxidative damage and inflammatory responses, curcumin delivery to these macrophages can result in its maximal anti-inflammatory action. Sou and colleagues (74) have reported localization of curcumin SLNs in macrophage-rich sites such as bone marrow, spleen, and liver even at 6 hours after the injection, showing their preferential uptake by macrophages and their considerable ROS scavenging potential equivalent to 160 to 1,050 superoxide dismutase (SOD) units when analyzed by a hypoxanthine and xanthine oxidase system (74). Although an initial decrease in white blood cells, red blood cells, and platelets was observed with these vesicular NPs, the levels of these blood components recovered within 3 hours showed absence of any acute toxic response of body toward these delivery vehicles. The potential of this system to deliver curcumin to different tissues was further shown by the presence of yellow fluorescence of curcumin in tissue samples of animals, as detected by confocal microscopy (Fig. 3; ref. 74). One concern with this approach, however, involves an increase in curcumin release from these vesicles at room

temperature (20°C–30°C), suggesting a possible problem with the retention of entrapped curcumin during long storage.

Liposomes

Liposomes are the spherical bilayer vesicles with an aqueous interior formed by the self-association behavior of amphiphilic phospholipids with cholesterol molecules. This self-associating behavior of phospholipids originates from their tendency to shield their hydrophobic groups from aqueous environment while interacting with the aqueous phase with their hydrophilic groups. Depending upon their bilayer structure and size, liposomes can be categorized as multilamellar, large unilamellar, or small unilamellar. Alternatively, depending upon the driving force for drug release, they can be classified as conventional liposomes, pH-sensitive liposomes, cationic liposomes, immunoliposomes, and long-circulating liposomes (reviewed in ref. 47). These lipid-based particulate carriers can significantly enhance the solubility of poorly water-soluble chemopreventives. Different drugs based upon their lipophilic character can distribute either in the phospholipid bilayer, in the interior aqueous phase, or at the bilayer water interface. The lipophilic nature of many chemopreventives including curcumin (Table 4), resveratrol (7, 75), oryzanol (76), and *N*-acetyl cysteine (77), make them suitable candidates for liposomal drug delivery where lipophilic core of these liposomes provide an optimum environment for drug entrapment (78).

A liposomal system for the targeted delivery [by coating with prostate-specific antigen (PSA) antibodies] of curcumin was also reported to study its partitioning potential (34). It has been observed that DMPC-based liposomes

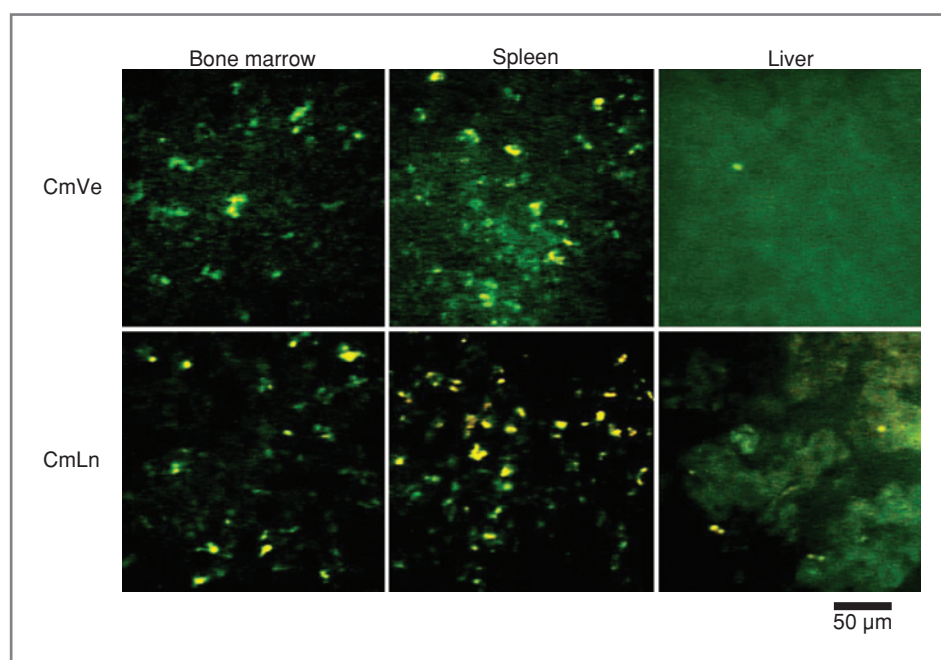


Figure 3. Confocal scanning images of bone marrow, spleen, and liver tissues of rats 6 hours after the intravenous administration of curcumin vesicles (CmVe) and curcumin lipid nanospheres (CmLn). Yellow fluorescence shows the presence of curcumin in the tissues [Reprinted with permission from Sou and colleagues (74)].

Table 4. Liposomal formulations of curcumin for parenteral administration

Formulation	Method	Particle size, nm	Encapsulation efficiency, %	Advantages	Refs.
DMPC:DMPG:cholesterol (7:1:8) liposomes of curcumin for parenteral administration.	Vortexing of SMVs	N.R.	N.R.	<ul style="list-style-type: none"> • Increased curcumin stability in PBS. • Increased efficacy in inhibiting ConA stimulated human lymphocytes and EBV transformed B cells. 	(80)
Pegylated DMPC, cholesterol, DMPG liposomes of curcumin for parenteral administration.	Extrusion through 0.22- μ m filter	N.R.	N.R.	<ul style="list-style-type: none"> • Significant growth inhibition of Colo205 and LoVo cells in nude xenograft mice. 	(79)
DMPC:DMPG (9:1) liposomes of curcumin for parenteral administration.	Extrusion through 0.22- μ m filter	N.R.	N.R.	<ul style="list-style-type: none"> • Significant growth inhibition of CAL27 cells in nude xenograft mice. 	(81)
DMPC liposomes coated with PSA-specific antibodies.	Sonication of SUVs	100–150	N.R.	<ul style="list-style-type: none"> • Increased inhibition of LNCaP and C4-2B cells at 10-fold lower doses of curcumin. 	(34)

Abbreviations: N.R., not reported; SMVs, small multilamellar vesicles; SUV, small unilamellar vesicles.

possess greater encapsulation efficiency with a more desirable particle size of 100 to 150 nm as compared with liposomes prepared with dipalmitoyl phosphatidylcholine (DPPC) and egg phosphatidylcholine (PC). Furthermore, DMPC liposomes were found to inhibit (70%–80%) cellular proliferation of the human prostate LNCaP and C4-2B cancer cells at 5 to 10 μ mol/L of concentration as compared with free curcumin that required 10-fold higher doses to elicit similar inhibition. Both *in vitro* and *in vivo* studies have shown that liposomal curcumin is much more effective than free curcumin at equimolar concentrations emphasizing that liposomal delivery of curcumin can enhance their uptake and hence bioavailability/activity into the cells (34). A liposomal formulation of curcumin using dimyristoyl-*sn*-glycero-3-phosphocholine was also tested for its effects on the modulation of signaling pathways involving proliferation, apoptosis, and angiogenesis of human pancreatic carcinoma cells (79). When administered at 40 mg/kg (3 times/wk), this liposomal formulation suppressed the growth of BXP3 and MiaPaCa2 tumors in a xenograft murine model suggesting *in vivo* efficacy of these liposomes (79).

Chemopreventives as liposomal formulations can also be delivered transcutaneously through hair follicles (82) providing a reservoir for locally applied substances and to enable topical administration. Jung and colleagues (82) investigated the penetration depth of a novel class of amphoteric liposomes having isoelectric point at slightly acidic pH to measure the efficiency of transfollicular delivery of curcumin. They found that these liposomes can

penetrate about 35% to 69% of the follicle length depending upon the charge on the liposomes, showing their ability for topical delivery of lipophilic chemopreventives for both therapeutic as well as chemopreventive purposes. However, rapid elimination of these liposomal vesicles by active opsonization is known to limit their overall efficacy which can be avoided by modifying the liposomal surface with polymers such as PEG to confer stealth properties to them. Similar liposomal delivery systems are also reported for active curcumin metabolites like tetrahydrocurcumin (THC). Government Pharmaceutical Organization of Thailand developed a THC cream formulation using phospholipid-derived THC liposomes (83). Dermatologic tests for irritation, carried out by Wattanakrai and colleagues (83) on human female volunteers, showed that these liposomes were not only safe but also possessed a significantly lower irritation potential compared with the reference material. Furthermore, a corneometry analysis of the skin above antecubital fossa revealed a higher moisturizing effect, which further showed that topical delivery of liposomal curcumin can be used in various skin ailments. However, some of the major problems of this delivery system include stability, poor batch to batch reproducibility, sterilization difficulties, and low drug loading (84).

Microemulsions/microencapsulation

Microemulsions are one of the most widely used drug delivery systems capable of providing high drug entrapment efficiency with long-term stability of hydrophobic molecules (85). These thermodynamically stable, optically isotropic, transparent formulations are characterized by

a dynamic microstructure that results spontaneously by mixing lipophilic and hydrophilic excipients in presence of suitable surfactants (86). This microstructure results in high drug solubilization capacity along with free and fast drug diffusion (87) that coupled with lipophilic nature endow them with a high potential for delivering lipophilic compounds like curcumin not only across lipophilic cell membranes but also through skin. Studies by Teichmann and colleagues (87) showed that curcumin can easily be delivered through the stratum corneum and into the complete follicular infundibula via o/w (oil in water) microemulsions. These microemulsions can be further formulated into hydrogel patches of chitosan or chitosan starch blends to protect the drug from the detrimental effects of pH-, light-, and/or oxygen-mediated degradation (88). Once these agents are microemulsified and entrapped into a hydrogel like matrix, their stability increases significantly and controlled release at a desirable site can be obtained. Studies have shown that even after 2 months of storage at room temperature, mean hydrodynamic diameter of the oily internal phase increases only slightly, showing the high stability and efficiency of such hydrogels (88). In addition, the external aqueous phase of these emulsions provides hydration to the stratum corneum and moisturizes the skin (88). Drug release from microemulsified droplets can be further augmented by using external energy sources such as ultrasonic waves. It has been observed that on application of external energy, these droplets undergo a structural reorganization that results in the phase separation of oil droplets from the aqueous vehicle releasing the compound (82). Similarly, a microemulsion cream formulation of curcumin SLNs was also described by Tiyaboonchai and colleagues (89). An entrapment efficiency of 35% to 70% was shown for curcumin in SLNs with a diffusion-mediated controlled release pattern. In addition, the formulation was found to increase the photostability of curcumin where even after 6 months of storage, no significant change in the viscosity or color of the formulation was observed (89). Although this approach seems promising in enhancing the delivery of potent therapeutics, its usefulness for chemopreventives has not been established in animal and human clinical studies.

Aziz and colleagues (90) prepared microcapsules of curcumin with gelatin using ethanol/acetone as coacervating agents to separate the 2 phases that result in precipitation of the drug in spherical microcapsules. They prepared curcumin dispersion in the gelatin solution followed by its addition to ethanol. A formaldehyde solution (37% v/v) was then added to provide rigidity to gelatin coating. It was reported that microencapsulation yield, drug loading, and entrapment efficiency all were significantly affected by the solubility of curcumin in the coacervating solvents. They were higher when acetone was used to dissolve curcumin as compared with ethanol in which curcumin tend to disperse at high concentrations used for loading into microemulsions. Furthermore, the microcapsules prepared by using acetone were found to possess better flowability and high stability with retention of their spherical shape (90). A similar inject-

able microparticulate formulation of curcumin using PLGA polymer was prepared and used in breast cancer chemoprevention study (91). These microparticles were found to provide sustained blood and tissue levels for around 1 month by a single subcutaneous injection with tissue levels 10-to 30-fold higher in brain and lung as compared with that in plasma, suggesting their potential to sustain drug levels on subcutaneous administration (Table 5).

Implantable drug delivery systems

Over the past few decades, polymeric implantable drug delivery systems have exhibited tremendous potential for systemic delivery of various therapeutic agents, including carmustine and leuprolide at a controlled rate (92, 93). These implants with homogeneous entrapment of drugs in a polymeric matrix achieve sustained localized delivery coupled with complete bioavailability into systemic circulation by slowly releasing the encapsulated drug at the site of implantation (94). Furthermore, due to their slow release kinetics, implants can provide drug release ranging from months to years which improves the patient compliance, especially for poorly bioavailable and rapidly metabolized compounds like curcumin (95).

There are 2 types of implantable drug delivery systems reservoir type and matrix type. In reservoir type implants, drug core is coated by a semipermeable polymeric membrane which controls the rate of drug release and is dependent upon the rate of water influx into the system (96). But the reservoir type implants are often discouraged due to their probability of dose dumping. Matrix type implants on the other hand contain uniformly distributed drug into the polymeric matrix (96). Depending upon the polymer degradation characteristics, they can be surface erosion type (degrade only at the surface) or bulk erosion type (slow uniform degradation in the bulk of the implant; ref. 93). Matrix type implants are devoid of any dose dumping phenomenon and provide desirable biphasic drug release mediated by diffusion. This biphasic release consists of a burst release followed by a slow controlled release. Initial burst release delivers the drug for distribution to a large volume, to rapidly reach the therapeutic concentration and a slow, controlled release maintains the therapeutic concentrations for prolonged periods of time (97).

Recently, we developed poly (ϵ -caprolactone) (PCL) implants using solvent evaporation coupled with melt extrusion technique for many natural compounds, including curcumin (98). Implants were prepared by dissolving PCL together with F68 (PCL:F68, 9:1) in dichloromethane (DCM) and drug in ethanol and mixing the 2 solutions with stirring to prepare a homogeneous solution. The solvents were then evaporated on a water bath maintained at 65°C for 1 to 2 hours. The semisolid residue was then concentrated overnight under vacuum to ensure complete removal of the solvents. The dried polymeric material was melted and extruded at 70°C into silastic tubing (internal diameter = 3.4 mm) attached to a syringe and then cut into desired lengths (Fig. 4A). These implants were optimized under *in vitro* conditions for drug release kinetics by

Table 5. Microparticle/microemulsion formulations of curcumin

Formulation	Method	Particle size	Encapsulation efficiency, %	Advantages	Refs.
Curcumin macrocapsules prepared with gelatin.	Coacervation method	80–90 nm	75.5	<ul style="list-style-type: none"> Improved stability coupled with controlled release. 	90
PLGA microparticles of curcumin for parenteral administration.	Emulsion, solvent evaporation	22 ± 9 nm	75	<ul style="list-style-type: none"> Sustained plasma curcumin levels for upto a month after a single subcutaneous injection. Enhanced efficacy as compared to powder curcumin. 	91
Curcumin microemulsion (o/w) formulated into a hydrogel matrix for topical application.	Surfactant-mediated microemulsification	45–50 nm	Not reported	<ul style="list-style-type: none"> Porous polymeric network providing controlled release. 	88
Microemulsified curcumin loaded SLNs for topical application.	Microemulsification	450 nm (PDI = 0.4)	70	<ul style="list-style-type: none"> Increases skin hydration. Increased photostability. 	89

incubating them in PBS supplemented with (10% v/v) bovine calf serum (to simulate extracellular fluid conditions) and by changing the media daily to measure the drug released. *In vitro* release studies showed a biphasic release pattern for curcumin where an initial burst release was observed for the first week from surface bound drug followed by a diffusion controlled release from inner layers of polymeric matrix (Fig. 4B; ref. 10). Measurement of residual curcumin in the implants (2-cm implant; 200 mg; 10% or 20% drug load) recovered from Sprague Dawley rats at the time of euthanasia showed an about 1.8- to 2-fold higher cumulative drug release over a period of 5 weeks compared with *in vitro* release but with similar release kinetics (Fig. 4C; ref. 10). These implants (2 cm, 200 mg, 10% drug load) released about 2.64 mg (13.2%) of curcumin in the first week with an average daily release of about 370 µg/d which dropped slowly to around 240 µg/d after 35 days of implantation (10). Other studies conducted in this laboratory showed that these implants are stable and can release up to about 0.2 mg curcumin per week even after 42 weeks of implantation (98).

This polymeric implant delivery system not only provided high local concentrations of curcumin but also enabled systemic delivery of curcumin and other phytochemicals such as green tea polyphenols, punicalagins, and diindolylmethane to various other organs of the body (98). These implants were found to deliver significantly higher levels of curcumin in the plasma, liver, and brain tissues compared with the oral delivery of curcumin (unpublished data). Analysis of liver tissue from Sprague Dawley rats implanted with two 2-cm curcumin implants (200-mg implant with 10% drug load) showed

presence of 60 ± 20 ng/g of curcumin after 4 days of implantation which dropped to 8–15 ng/g after 7 days and stayed almost constant over a period of 5 weeks (10). Furthermore, curcumin delivered via the implant route was found to inhibit B[a]P-induced tissue DNA adducts, showing the biological efficacy of systemic delivery of these chemopreventives at substantially reduced (25- to 50-fold) doses compared with the traditional oral route (99). In summary, this novel, continuous release ("24/7") implant delivery concept has been found to be (i) applicable for many commonly used chemopreventive agents of varied lipophilicities; (ii) circumvent bioavailability issues for many of these agents compared with their administration by the traditional oral route; and (iii) enable minor components of plant origin to be tested *in vivo* for their chemopreventive/chemotherapeutic potential, which otherwise remain uninvestigated because of the high quantities required for oral delivery.

Bioavailability Issues for Other Chemopreventives

Although in this review, we have focused on oral bioavailability issues for curcumin and the advanced delivery systems that can enhance its bioavailability, the oral bioavailability problem has also been encountered for many naturally occurring chemopreventives, for example, EGCG, resveratrol, and ellagic acid, and the delivery systems described for curcumin can be readily adopted for these and other compounds. The natural compounds like EGCG, resveratrol and ellagic acid that showed efficacy in cell

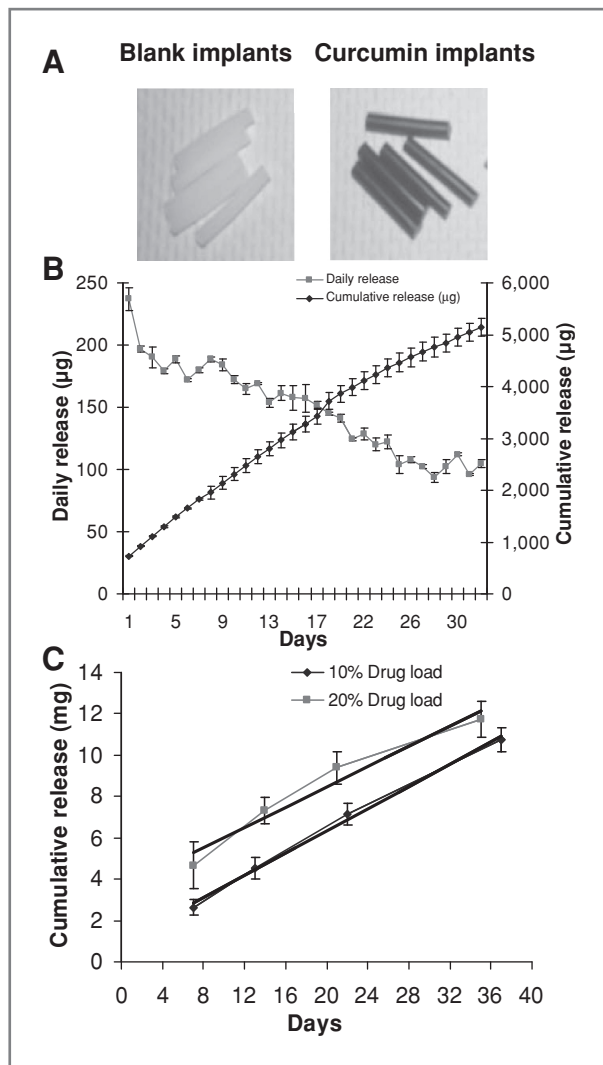


Figure 4. A, photographs of blank implants and of curcumin implants. B, *in vitro* release of curcumin from a 2-cm implant (3.4 mm diameter; 200 mg) with 10% curcumin load ($n = 3$). C, *in vivo* release of curcumin from a 2-cm implant formulated either with 10% or 20% curcumin load ($n = 3$). Implants were grafted subcutaneously at the back of Sprague Dawley rats and were recovered at the time of euthanasia at indicated times to measure the residual curcumin content. Cumulative release was calculated by subtracting residual amount at each interval from initial amount of drug present in implants [Reprinted with permission from data published by Bansal and colleagues (10)].

culture studies elicited limited activity in several animal studies. These compounds possess poor biopharmaceutical properties with low oral bioavailability, limited either by poor aqueous solubility and/or permeability for absorption into the systemic circulation. As a result, the advanced drug delivery systems like NPs [ellagic acid (ref. 5), resver-

atrol (ref. 100), EGCG (ref. 4), and quercetin (ref. 101)], liposomes [resveratrol (7), EGCG (ref.6), quercetin (ref. 102), and β carotene (ref. 103)], microparticles [quercetin (ref. 8), EGCG (ref. 9), and resveratrol (ref. 104)], and polymeric implants [EGCG (ref. 105), resveratrol, punicalagans, diindolylmethane, withaferin A, tanshinone II, etc.; our unpublished data] have been developed to circumvent their bioavailability issues.

Conclusions

Since ancient times, plant-derived compounds that are known to possess a plethora of activities have been used in the treatment and prevention of many ailments including cancer. However, even after half a century of research, none of the naturally occurring compounds (including the most investigated ones such as curcumin and EGCG) have managed to find a place in the physician's armamentarium for prophylactic treatments. The main reasons for their limited success in the clinical setting are their poor bioavailability, rapid rate of metabolism, or both. The quest to utilize traditional natural compounds for their chemotherapeutic and chemopreventive potential in the clinical setting has motivated drug delivery scientists to devise advanced drug delivery systems such as NPs, liposomes, microemulsions, and implants. Although research on most of these delivery systems has shown the potential for enhanced bioavailability, two important aspects would need attention: (i) the occurrence of rapid drug metabolism (e.g., for curcumin), which may be mitigated by the application of combination therapies such as with enzyme inhibitors like piperine, and (ii) the need for frequent parenteral dosing, to maintain effective therapeutic concentrations in the blood. It is now clear that further development of naturally occurring compounds with chemopreventive/chemotherapeutic potential will be dictated by the development of formulations that can bypass their poor oral bioavailability along with eliminating hepatic first-pass metabolism while not compromising with patient acceptability.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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