Controlled Systemic Delivery by Polymeric Implants Enhances Tissue and Plasma Curcumin Levels Compared with Oral Administration

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Abstract

Curcumin possess potent anti-inflammatory and anti-proliferative activities but with poor biopharmaceutical attributes. To overcome these limitations, curcumin implants were developed and tissue (plasma, brain and liver) curcumin concentrations were measured in female ACI rats for 3 months. Biological efficacy of tissue levels achieved was analyzed by modulation of hepatic cytochromes. Curcumin implants exhibited diffusion-mediated biphasic release pattern with \( \sim 2 \)-fold higher \textit{in vivo} release as compared to \textit{in vitro}. Plasma curcumin concentration from implants was \( \sim 3.3 \) ng/ml on day 1 which dropped to \( \sim 0.2 \) ng/ml after 3 months whereas only \( 0.2–0.3 \) ng/ml concentration was observed from 4–12 days with diet and was undetected subsequently. Almost 10 fold higher curcumin levels were observed in brain on day 1 from implants compared with diet (30.1\( \pm \)7.3 vs 2.7\( \pm \)0.8 ng/g) and were higher even after 90 days (7.7\( \pm \)3.8 vs 2.2\( \pm \)0.8 ng/g). Although, curcumin levels were similar in liver from both the routes (\( \sim 25–30 \) ng/g from day 1–4 and \( \sim 10–15 \) ng/g at 90 days), implants were more efficacious in altering hepatic CYP1A1 levels and CYP3A4 activity at \( \sim 28 \) fold lower doses. Curcumin implants provided much higher plasma and tissue concentrations and are a viable alternative for delivery of curcumin to various organs like brain.

Keywords
Curcumin; Implants; Bioavailability; Chemoprevention; Controlled Release; Tissue curcumin levels

Introduction
Curcumin, a polyphenolic chemopreventive is known to possess potent anti-oxidant, anti-inflammatory, anti-proliferative, ant-metastatic, anti-diabetic and various other activities

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Conflict of Interest Statement
The authors declare that there are no conflicts of interest.
It is a mixture of 3 curcuminoids; curcumin (curcumin I), demethoxycurcumin (curcumin II) and bis-demethoxycurcumin (curcumin III) isolated from rhizomes of *Curcuma longa* (turmeric) [3]. Its chemopreventive and chemotherapeutic activities have been ascribed to its potential to (i) favorably induce various phase I cytochromes involved in metabolism and excretion of various carcinogens [4, 5] (ii) induce phase II enzymes like GST and increase endogenous antioxidant levels [6], and (iii) inhibition of NF-κB activation by inhibiting IκB kinase [7, 8].

However, its non-optimal biopharmaceutical attributes like poor aqueous solubility and rapid metabolism results in its poor oral bioavailability and hinder its development as a therapeutic candidate [9]. Therefore, various advanced drug delivery systems like nanoparticles [10], microparticles [11], nano/microemulsions [12], liposomes [13] and phospholipid mixtures [14] have been prepared to harness complete potential of curcumin’s therapeutic activities [9]. Although some of these delivery systems were found to provide certain advantages over dietary consumption, their frequent dosing and low drug loading capabilities limit patient compliance. To improve patient compliance as well as to achieve higher loading, we developed and optimized curcumin implants [15] using polycaprolactone polymer to improve its drugability. These implants can be grafted subcutaneously to continuously (“24/7”) deliver the drug directly into the systemic circulation for months to years. We showed that these implants can deliver on an average 200–300 µg of curcumin daily from a single implant sufficient to achieve significant liver curcumin concentrations (~0.1 µM) that can modulate liver cytochromes [15]. Furthermore, we also showed that continuous systemic administration of curcumin was not found to induce any systemic toxicity as measured by liver and kidney function [16]. Therefore, this study was designed to further evaluate and compare this implantable drug delivery system of curcumin with traditional route of oral administration with which most of the animal and clinical studies have been conducted. In this study, we compared the tissue levels of curcumin achieved via these implants compared with the traditional dietary route at a dose of 1000 ppm. Furthermore, biological efficacy of curcumin to alter expression and/or activity of various hepatic xenobiotic metabolizing enzymes was also determined and compared by these routes of delivery.

**Materials and Methods**

**Materials**

Medical grade poly (ε-caprolactone) 121,000 molecular weight (PCL-121) was purchased from SurModics pharmaceuticals (Birmingham, AL), dichloromethane, acetonitrile, anhydrous citric acid and phosphate-buffered-saline (PBS) were from Sigma-Aldrich (St. Louis, MO), polyethylene glycol of 8,000 molecular weight (PEG-8K) from Fisher Scientific (Fair Lawn, New Jersey), ethanol from Pharmco-AAPER (Louisville, KY), bovine calf serum (BCS) was from Hyclone (Logan, UT) and silastic tubing (3.4 mm internal diameter) from Allied Biomedical (Ventura, CA). Curcumin (C-3 complex) extracted under GMP conditions was a generous gift from Sabinsa Corporation (East Windsor, NJ). All the materials were used as received without any further analysis.

**Methods**

**Preparation of Curcumin Implants**—Curcumin implants were prepared by following the procedure described elsewhere [15]. Briefly, curcumin (20% w/w) was dissolved in ethanol and polymers (PCL-121 and PEG-8K in 65:35 ratios) were dissolved in dichloromethane. Both solutions were then mixed together to prepare a homogenous solution of drug and polymers. The solvents were evaporated on a water bath maintained at 65 °C followed by overnight drying under vacuum at 65 °C to remove residual solvents to
prepare an amorphous molecular dispersion of drug in polymer. The dried molten polymer was then extruded through silastic tubing mold (internal diameter 3.4 mm) attached to a syringe. After few min, the cylindrical implants were removed from the tubing and excised into desired lengths (2.0 cm, 200 mg containing 40 mg drug) and stored at −20 °C under argon until used.

**In Vitro Release Studies**—*In vitro* release of curcumin from implants was studied under simulated conditions by using 10 ml phosphate-buffered-saline (PBS) (pH 7.4) supplemented with 10% (v/v) bovine calf serum (BCS) in amber colored vials to reduce light and pH mediated degradation [15]. PBS was supplemented with BCS (10% v/v) to mimic extracellular fluid composition and to simulate the *in vivo* situation. Amber colored vials (n=3) containing a 2-cm implant each were incubated at 37±0.5 °C in a reciprocating (150 rpm) shaker water bath (Julabo SW23, Seelbach, Germany) and the release medium was changed after every 24 h. To the collected medium, 1 ml ethanol was added to solubilize any precipitated drug and curcumin concentration was measured spectrophotometrically at 430 nm using SpectraMax M2 Microplate Reader (Molecular Devices, Sunnyvale, CA).

**In Vivo Studies**—*In vivo* cumulative release was studied in female ACI (Augustus Copenhagen Irish) rats following an approved protocol from the Institutional Animal Care and Use Committee (IACUC). All the animals were randomized into 4 groups as: 1) untreated (no intervention, provided with control diet; AIN-93M), 2) sham implants treated (two 2-cm blank implants prepared without curcumin), 3) two 2-cm curcumin implants, and 4) curcumin diet. Curcumin diet was prepared and supplied as pellets by Harlan Laboratories, by mixing curcumin (at 1,000 ppm). All the animals were provided food and water *ad libitum*. Sham and curcumin implants were subcutaneously grafted at the back of the rats as described previously [17] and were monitored for their diet intake and body weight. Animals were euthanized at different time intervals by asphyxiation to collect blood, liver, brain, local tissues and implants. All the tissues were snap frozen in liquid nitrogen and stored at −80°C until further analysis. Implants were dried overnight under vacuum, weighed and stored at −80°C until analysis of the residual curcumin content.

**Residual Drug Determination/Cumulative Drug Release**—Initial and residual curcumin content in implants (n=3) was analyzed to determine the cumulative drug release. Implants recovered from animals were dried over night under vacuum and dissolved in 5 ml dichloromethane. Once the implants were dissolved, 5 ml ethanol was added to completely dissolve the curcumin. The solution was then diluted suitably (1:10) with ethanol containing 20% DCM followed by in ethanol (1 in 40). Drug concentration was measured by UV spectrophotometer at 430 nm to calculate the amount of curcumin released *in vivo* using following equation:

\[
\text{Average daily release} = \frac{\text{Residual amount at } T_n - \text{Residual amount at } T_{n-1}}{T_n - T_{n-1} \text{ (Days)}}
\]

where T₁, T₂, T₃……Tₙ are different time intervals.

**Analysis of Plasma and Tissue Curcumin Levels**—Plasma pooled from all the animals (1.5 ml) was mixed with 200 µl of 0.5 M sodium acetate to reduce the pH to 5. Plasma was then extracted thrice with 3 ml ethyl acetate. Ethyl acetate extracts were pooled and dried under vacuum. The dried residue was reconstituted in 100 µl of acetonitrile and one half of the solution was analyzed by HPLC. Similarly, liver or brain tissue (~500 mg) from each animal was homogenized in 3 ml PBS (pH 7.4) containing 200 µl sodium acetate.
The homogenate was then extracted twice with 2 volumes of ethyl acetate. After evaporation of ethyl acetate under vacuum, the residue was reconstituted in 1 ml acetonitrile. The solution was filtered through 0.45 µ glass-microfiber filter and evaporated again under vacuum. The residue was finally reconstituted in 100 µl acetonitrile and one half of it was analyzed by HPLC using Shimadzu liquid chromatography system equipped with LC-10AD VP pump, RF-10A XL fluorescence detector and a Shimadzu C18 column of 5 µm particles (250 × 4.6 mm). The three curcuminoids were separated by using acetonitrile and 1% citric acid (adjusted to pH 2.5) at a flow rate of 1 ml/min with a gradient elution in which acetonitrile concentration was increased from 0 to 30% in first 5 min, followed by an increase to 45% in next 5 to 20 min. Acetonitrile was then maintained at this ratio till 36 min. Curcuminoids were detected using 410 and 500 nm as excitation and emission maxima, respectively, in the fluorescence detector.

**Microsome Extraction**—Liver (~100 mg) was homogenized in 0.25 M sucrose buffer (pH 7.4) at 3,000 rpm with Polytron. The homogenate was centrifuged at 3,000g for 20 min at 4 °C to separate the nuclear content. The supernatant was collected and again centrifuged at 11,000g for 20 min at 4 °C to separate the mitochondria. The post-mitochondrial supernatant was then transferred to ultracentrifuge tubes and finally centrifuged at 100,000g for 1 h at 4 °C to separate the microsomes. The pellet was suspended in 1 ml sucrose buffer, aliquoted and stored at −80 °C until use.

**Analysis of Cytochrome P450s and GSTµ (GSTM)**—Microsomal proteins were quantified using bicinechonic acid (BCA) method [18] using BCA™ Protein Assay kit (Thermo Scientific, Rockford, IL) and were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% gel. Protein bands were then transferred to polyvinylidene difluoride (PVDF) membrane which was incubated with 5% non-fat dried milk in Tris-buffered-saline for 1 h at room temperature (25 °C) to block the non-specific binding sites. The membrane was then incubated with primary antibodies for CYP1A1 and GSTM, followed by horseradish peroxidase conjugated secondary antibody. The bands were detected by enhanced chemiluminescence using Pierce® ECL Western-Blotting Substrate (Thermo Scientific, Rockford, IL) and quantified using VersaDoc Imaging System (BioRad Laboratories, Hercules, CA).

**Results and Discussion**

Recently we demonstrated the development and in vitro-in vivo evaluation of curcumin implants prepared using polycaprolactone and F-68 (90:10) with 10% drug load (10 mg/cm implant) [15]. Further optimization studies showed that replacement of F-68 (10% w/w) with PEG-8K (35% w/w) significantly enhances drug release both during the initial burst phase as well as during the controlled release phase [16]. Furthermore, these implants were found to be safe and biocompatible for at least a period of 3 months without any effects on physical wellbeing of the animals. We showed that these curcumin doses administered by the implants were safe and did not induce any biochemical changes in liver and kidney function [16]. Therefore, this study was designed in continuation to above described work (i) to analyze the in vivo release from these implants, (ii) to compare the tissue distribution of curcumin delivered via these implants with curcumin delivered via diet; and finally (iii) to compare the efficacy of curcumin delivered via both routes.

**In-vitro Release Kinetics**

In vitro release studies were performed under simulated conditions using 10 ml PBS supplemented with 10% BCS to mimic extracellular fluid composition (Fig 1). Curcumin release was measured in whole release media by replacing it with fresh media after every 24 hours.
hrs. As was described earlier [15], these implants were found to exhibit diffusion mediated Higuchi kinetics ($r^2 > 0.97$) with biphasic release pattern under in vitro conditions. Initial burst phase was observed for first 7–8 days with a release of ~570 µg of curcumin on day 1 that decreased rapidly to ~390 µg on day 2 before reducing to ~200 µg/day after 1 week. A controlled release was observed in the 2nd phase where rate of drop in drug release was relatively lower and release was ~120 µg/day even after 31 days (Fig 1). This initial burst release was due to rapid dissolution of surface bound drug into the release media and once the drug release started from inner layers of polymeric matrix, a more controlled release was observed in the 2nd phase [19]. Furthermore, cumulative release was found to be ~15% of total drug load (40 mg per implant) after 1 month.

**In vivo Release Kinetics**

Cumulative in vivo release was determined by measuring the residual drug content in implants grafted and recovered from ACI rats after specific time intervals (Fig 2.). In vivo release was observed to mimic the in vitro release with a burst release of ~1,800 µg on day 1 which rapidly declined to ~380 µg on day 4 after which more stable release kinetics were observed. Drug release was found to be 300–350 µg/day for over a period of 1 month and then reduced to ~130 µg/day after 3 months. Cumulative in vivo release was ~30% after 1 month which was ~1.8–2 fold higher than in vitro release suggesting a good correlation between simulated in vitro and in vivo conditions. Furthermore, only 47% (19 mg out of 40 mg) drug was found to be released from these implants over a period of 3 months showing their potential to deliver curcumin for extended periods of time (perhaps a year or longer).

Moreover, this release was higher than what we previously reported [15] with PCL implants containing 10% (w/w) either F-68 or PEG-8000. This higher release was due to inclusion of 35% PEG-8000 which being water soluble not only provides a more porous microstructure but also increases the hydrophilicity of the polymeric matrix. Weight loss studies showed that these implants lost a weight of ~36.3 mg after 1 day of implantation (supplementary Fig S-1). Since, PCL is a semi crystalline polymer with long half-life of degradation (2–3 years), this weight loss was assumed to be due to release of PEG (34.5 mg) and curcumin (1.8 mg) (PEG-8000 release was measured by subtracting the amount of released curcumin and weight of dried implant recovered from animals from initial weight of the implants).

**Tissue Distribution of Curcumin**

As described previously [15], during in vivo release extracellular fluid from the site of implantation enters into the polymeric matrix, dissolves the drug and diffuses out into the surrounding tissue. Curcumin from surrounding tissues then enters into the systemic circulation and is distributed to different tissues. Therefore, we measured tissue concentrations of curcumin delivered via both the diet and implants in plasma, liver and brain. Curcumin was extracted from the tissues using solvent extraction and analyzed using HPLC coupled with a fluorescence detector with an extraction efficiency of ~90 % from plasma and ~70% from tissues (liver and brain). Fluorescence detector was used due to very high fluorescence of curcumin which not only increased the specificity of curcumin detection but also increased the sensitivity by at least 4–5 fold. The lower limit of detection in plasma was found to be 125 pg (340 pM) and limit of quantification was ~200 pg (540 pM) by this method.

When ~500 µl of plasma was extracted from each individual animal and analyzed by HPLC, the levels were found to be very low and were below the quantification limit of the method. Therefore, to increase the specificity and reliability of quantification, the extraction was performed with 1.5 ml plasma by pooling 250–350 µl of plasma from all the animals (4–6 in each group) (Fig. 3). The plasma curcumin levels from the implants group were found to be
3.3 ng/ml (9 nM) on day 1 which decreased rapidly to 1.4 ng/ml (4 nM) on day 4 after which a slow and steady decrease in plasma concentration was observed reaching ~0.2 ng/ml (543 pM) after 3 months. Curcumin diet on the other hand, showed different kinetics in that curcumin was detected only on day 4 at a level of 0.3 ng/ml (815 pM) decreasing to 0.2 ng/ml after 12 days (543 pM) and was undetectable on 25, and 90 days. It was highly counter intuitive that instead of the fact that curcumin diet consumption was constant at all the time periods, curcumin was not observed on day 1 and a decline in plasma curcumin concentrations was observed after day 4, suggesting time- and exposure-dependent absorption kinetics. Furthermore, curcumin due to its lipophilic nature exhibits biphasic elimination kinetics with rapid equilibration and distribution of plasma curcumin into various tissues [20]. It is, therefore, also possible that due to high tissue distribution of curcumin, initially almost all curcumin equilibrates into tissues and was not observed in plasma on day 1. However, with time as the lipophilic tissues gets saturated, curcumin was observed in plasma after 4 days on dietary administration.

It is known that curcumin possess poor oral bioavailability owing to its low aqueous solubility [21] and rapid metabolism both in intestine as well as in liver [22, 23]. Therefore, to further understand the kinetics of curcumin delivered via both the routes, its concentration in liver was also measured (Fig. 4). Curcumin delivered via both routes was found to be at similar levels in liver and was 25–30 ng on day 1. The levels increased slightly, though insignificant, on day 4 after which the levels declined to 10–15 ng after 12 days. This trend was found to exactly mimic the plasma concentrations suggestive of hepatic regulation of plasma curcumin levels at least via dietary route. Curcumin is known to induce the expression and activity of various enzymes like CYP1A1 [24], GSTM [25], CYP3A4 [26] and it appears that significant amounts of curcumin that reaches the liver tissue from days 1–4, induce its own metabolism by increasing the expression/activity of these xenobiotic-metabolizing enzymes. The levels, therefore, decreases significantly after 12 days and stayed almost constant afterwards. However, it is to be noted that curcumin concentration was 1000 ppm in the diet which corresponds to around 900 mg delivered over a period of 90 days (or 50 mg/kg per day considering an average weight of 200 g per rat) as opposed to only 38 mg delivered by both the implants combined. Furthermore, curcumin concentration was found to be much higher (10–20 fold) in liver as compared to plasma via both routes again suggestive of its high tissue distribution. High curcumin concentrations in hepatic tissues via dietary administration were expected as most of the orally administered curcumin gets absorbed into the liver where it undergoes rapid metabolism. However, similar behavior from the implant route was unexpected as it bypasses the hepatic first-pass metabolism and almost all of the administered curcumin directly reaches into the systemic circulation. Therefore, a much higher concentration was expected in plasma from implant route. It appears that such a pharmacokinetic behavior also originated from curcumin’s high lipophilicity resulting in high tissue distribution as compared to plasma. Moreover, since physiologically liver is a highly perfused organ and gets around 23% of total cardiac output, it is highly probable that most of the curcumin that reaches into the systemic circulation might get rapidly equilibrated and entrapped into the liver tissue. An almost constant liver concentration of curcumin from 12 to 90 days also suggests tissue binding of this compound.

To support these observations, we further analyzed curcumin concentration in the whole brain tissue as it is also one of the highly perfused organs. Brain was also selected because of curcumin’s known potent activities against Alzheimers disease [27] and against brain gliomas [28] where continuous localized/systemic delivery of this compound could make a significant improvement in the life of such patients.

Analysis of brain tissue showed almost constant levels of curcumin (2–3 ng/g) from dietary administration at all the time points (Fig 5.). Although, curcumin was not found in plasma
on day 1 in the dietary curcumin group, it indeed was present in brain. This observation further supports the fact that initially all the curcumin that escaped from liver and reached the systemic circulation partitioned into highly lipophilic sites like liver and other tissues. However, as equilibrium is established between plasma and other organs like brain (through blood brain barrier), curcumin started to accumulate in plasma and could be detected after 4 days of oral administration. Furthermore, constant curcumin levels in brain detected even at 90 days again suggests increased metabolism of curcumin in liver tissues that led to decreased curcumin concentration in liver at 12 days.

On the other hand, curcumin delivery to the brain via implant route showed biphasic kinetics similar to liver. Since drug concentration that reaches a tissue is determined by rate and extent of its perfusion by systemic circulation, brain showed rather high curcumin levels (30±7.3 ng/g tissue), almost equal to liver concentration on day 1 of implantation. This concentration dropped slowly to around 7.26±2.45 ng/g tissue after 12 days and stayed almost at the same levels even after 90 days. It is to be noted that the brain concentration was slightly less than the liver concentration from 12 to 90 days (a constant drug concentration period). It is known that physiologically, liver gets ~23% of cardiac output and brain gets slightly less than liver (~18%). This fact again shows that tissue distribution from implant route was indeed perfusion rate limited and was dictated by cardiac output to that particular organ. It may also be emphasized that curcumin concentration in the brain tissue was ~3.5 fold higher by the implant route in which case total curcumin administered was nearly 25 fold lower compared to oral administration over a period of 3 months.

Analysis of curcumin in different tissues showed that plasma curcumin concentration via dietary administration is regulated by liver. At high liver concentrations, a fraction of dietary curcumin escapes hepatic first pass metabolism, enters the systemic circulation and gets distributed to other organs. However, this is not the case with the implant route where plasma is the first site of curcumin absorption from where it gets simultaneously distributed to all the other tissues. Tissue concentrations are, therefore, determined only by the extent and rate of blood that supplies the organ. In view of these facts, it is possible that to administer curcumin to other organs like prostate or pancreas which are not richly supplied with blood, dietary administration might not be an appropriate route. In such cases, direct systemic administration either via parenteral routes or via implant route would be a better alternative that can be further evaluated for various preclinical and clinical studies.

Modulation of Phase I and Phase II enzymes

CYP1A1 and GSTM (µ) Expression Analysis in Hepatic Microsomes—Various in vitro and cell culture studies showed that curcumin’s effective concentration is 5–20 µM depending upon the cell line used [3]. Since the plasma and liver concentrations observed in our in vivo study (78–95 nM) were much below these; we also determined if these concentrations were effective to modulate hepatic cytochromes. Studies have shown that curcumin is a natural agonist of aryl hydrocarbon receptor (AhR)/pregnane xenobiotic receptor (PXR) pathway and induces the expression of phase I enzymes like CYP1A1 [24] and CYP3A4 [26]. Furthermore, it also interacts with the keap1 protein to lower its affinity for nuclear factor (erythroid-derived 2)-like 2 (Nrf2) protein followed by Nrf2 transloaction into the nucleus to induce phase II enzymes like glutathione-S-transferase(µ) (GSTM) [25]. Therefore, we isolated hepatic microsomes and analyzed them for their CYP 1A1, CYP3A4 and GSTM levels (an inducible cytochrome) by western blotting and activity of CYP3A4 (a major drug metabolizing enzyme) after 1, 4, 12, 25 and 90 days of curcumin treatment by both dietary and implant routes.

As is evident from the Fig. 6, curcumin implants were found to induce expression of CYP1A1 by ~2-fold after 4, 12, 25 and 90 days of treatment. Although, a 2-fold increase in
CYP1A1 was observed on day 1 also, yet the effect was masked by PEG-8000 as a similar effect was also observed in sham implants (blank implants prepared with only PCL-121 and PEG-8K without any drug) at this time point and this effect from the sham implants was absent at all other time points. This effect from sham implants was presumably due to release of significant amounts of PEG-8000 [29] on the first day (~34.5 mg) which decreased significantly with only 2.2 mg/day from 2 to 4 days (total ~6.5 mg over 3 days) (Supplementary Fig S-1). Curcumin diet, on the other hand, increased the CYP1A1 levels only slightly on day 1 (1.43 fold) which returned to basal levels after 4 days and infact, the levels were downregulated after 25 and 90 days of treatment. Since, the hepatic curcumin levels were similar by both the routes tested; such route-dependent differences in induction of CYP1A1 expression were counter intuitive. It is known that various chemopreventives like indole-3-carbinol (I3C) are most effective in inducing CYP1A1 when given orally as compared to when administered systemically. It has been found that polymerization products of indole-3-carbinol like diindolylmethane (DIM) formed under stomach acidic conditions are more effective AhR ligands as compared to the parent compound, thus resulting in higher efficacy of indole-3-carbinol [30]. Curcumin is also known to degrade under slightly basic conditions of intestine forming various active degradation products like ferulic acid and vanillin [31]. It is possible that one or more of these metabolites might have AhR antagonistic activity resulting in blunting of curcumin’s CYP1A1 upregulating activity at initial time points and eventual downregulation after 25 and 90 days of treatment. GSTM, on the other hand was not found to get modulated via either of the routes (data not shown).

**CYP3A4 Activity and Expression Analysis in Hepatic Microsomes**—Effect of curcumin on CYP3A4 activity and expression was also measured as it is the major drug metabolizing enzyme known to get induced by curcumin (Fig. 7). As was observed with CYP1A1 expression, CYP 3A4 activity was also found to be slightly increased by sham implants both at day 1 and day 4 but became significant only after 12 days of treatment which again dropped to basal levels by 25 days. From the weight loss data (Supplementary Fig S-1), it is evident that from 1–12 days, there was a weight loss of ~20 mg out of which 13.4 mg was from loss of PEG-8K. It, therefore, appears that low concentrations of PEG (~1.0 mg/day) which were insufficient to increase CYP1A1 expression after day 1 were sufficient to increase the CYP3A4 activity.

Similar to sham implants, curcumin implants were also found to increase the activity from day 1 to day 12 of treatment as compared to untreated animals; however, this increase became significant only after 25 days when compared with sham implants. This enhanced activity remained consistently up-regulated even after 3 months of treatment (in contrast to sham implants) and did not reduce to basal levels after 25 days. On the other hand, curcumin diet showed slightly higher activity on day 1 of treatment which was significant higher after 4 days as compared to untreated animals and again reduced to basal levels at 12 days of treatment. It is, therefore, evident that continuous delivery of curcumin directly into the systemic circulation by the implant route was more effective in increasing and maintaining the higher CYP3A4 activity as compared to dietary route.

**Conclusions**

Polymeric implants are a viable alternative for delivery of curcumin to circumvent its bioavailability problem by the traditional oral route and to harness its complete therapeutic potential. These studies showed that subcutaneous polymeric implants can provide a controlled release of curcumin at the local site from which it gets absorbed into the systemic circulation and then is distributed to all the tissues simultaneously. These implants were found to provide higher curcumin concentrations in plasma, brain and to some extent in liver over a period of 3 months as compared to dietary administration. The higher plasma and
tissue levels by implants were achieved by a reduction of curcumin dose by nearly 20–25 fold for over a period of 3 months as compared to the traditional dietary route. Furthermore, curcumin delivered directly into the systemic circulation was found to be more efficacious in inducing CYP1A1 and CYP3A4 enzymes required for its chemopreventive activity against various environmental carcinogens. The presence of significantly higher concentrations of curcumin in tissues like brain shows potential of this system for patients suffering with Alzheimer’s disease and/or brain gliomas where curcumin has shown significant potential in various in situ and cell culture studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

- PCL-121: poly (ε-caprolactone) of 121,000 molecular weight
- PEG 8K: polyethylene glycol (molecular weight 8,000)
- DCM: dichloromethane
- CYP1A1: cytochrome P450 1A1
- CYP3A4: cytochrome P450 3A4
- GSTM: glutathione S-transferase (µ)
- PBS: phosphate-buffered-saline
- BCS: bovine calf serum

References


Fig. 1.
Daily and cumulative drug release of curcumin from a 2-cm implant (20 mg/cm drug) under simulated in vitro conditions (n=3).
Fig. 2.
Average daily release of curcumin from a 2-cm implant containing 40 mg of drug under *in vivo* conditions. Implants were subcutaneously grafted at the back of the ACI rats, recovered after indicated time intervals and residual curcumin was measured to determine cumulative drug release.
Fig. 3.
Curcumin levels in the plasma of ACI rats treated with curcumin implants (two 2-cm implants: 20 mg curcumin/cm) or curcumin diet (1,000 ppm). One and half ml plasma was extracted from each time point after pooling and analyzed by HPLC using fluorescence detector as described under methodology.
Liver curcumin levels in ACI rats treated with curcumin implants (two 2-cm implants, 20 mg/cm implant) or curcumin diet (1,000 ppm). Based on around 10 g daily diet consumption, total curcumin administered over 90 days corresponded to 900 mg (or ~50 mg/kg) as opposed to 38 mg delivered by both the implants combined. Tissue (~500 mg) from individual animal (n = 4–6) was extracted and analyzed by HPLC coupled with a fluorescence detector as described in methodology.
Fig. 5.
Brain curcumin levels in ACI rats treated with curcumin implants (two 2-cm implants, 20 mg/cm) or dietary curcumin (1,000 ppm). Tissue (~500 mg) from individual animals was extracted and analyzed by HPLC coupled with a fluorescence detector. Data represent average of 4–6 animals, except for day 1 where data represents average of two animals since curcumin was below the detection limit in the remaining animals.
Fig. 6.
Effect of curcumin administered by polymeric implants (two 2-cm implants, 20 mg/cm) or diet (1,000 ppm) on CYP1A1 protein expression in hepatic microsomes of ACI rats. Sham implants (blank implants prepared without curcumin) and curcumin diet groups were compared with untreated controls and curcumin implants were compared with sham implants for all statistical purposes at a significance level of p value <0.05 (n=3–4).
Fig. 7.
Effect of curcumin administered by polymeric implants (two 2-cm implants, 20 mg/cm) or diet curcumin (1,000 ppm) on CYP3A4 activity in hepatic microsomes of ACI rats. Sham implants (Blank implants prepared without curcumin) and curcumin diet groups were compared with untreated controls and curcumin implants were compared with sham implants for all statistical purposes at a significance level of p value <0.05 (n=3–4).