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Research Paper

Novel formulation of solid lipid microparticles of curcumin for anti-angiogenic and anti-inflammatory activity for optimization of therapy of inflammatory bowel disease

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Abstract

Objectives This project was undertaken with a view to optimize the treatment of inflammatory bowel disease through a novel drug delivery approach for localized treatment in the colon. Curcumin has poor aqueous solubility, poor stability in the gastrointestinal tract and poor bioavailability. The purpose of the study was to prepare and evaluate the anti-inflammatory activity of solid lipid microparticles (SLMs) of curcumin for the treatment of inflammatory bowel disease in a colitis-induced rat model by a colon-specific delivery approach.

Methods We have developed a novel formulation approach for treating experimental colitis in the rat model. SLMs of curcumin were prepared with various lipids, such as palmitic acid, stearic acid and soya lecithin, with an optimized percentage of poloxamer 188. The SLMs of curcumin were characterized for particle size, drug content, drug entrapment, in-vitro release, surface morphology and infrared, differential scanning calorimetry and X-ray studies. The colonic delivery system of SLM formulations of curcumin were further investigated for their anti-angiogenic and anti-inflammatory activity using chick embryo and rat colitis models.

Key findings Particle size, drug content, drug entrapment and in-vitro release studies showed that formulation F4 containing one part stearic acid and 0.5% surfactant had the smallest diameter of 108 μm, 79.24% entrapment and exhibited excellent in-vitro release characteristics when compared with other formulations and pure curcumin. SLMs of curcumin (F4) proved to be a potent angio-inhibitory compound, as demonstrated by inhibition of angiogenesis in the chorioallantoic membrane assay. Rats treated with curcumin and its SLM complex showed a faster weight gain compared with dextran sulfate solution (DSS) control rats. The increase in whole colon length appeared to be significantly greater in SLM-treated rats when compared with pure curcumin and DSS control rats. An additional finding in the DSS-treated rats was chronic cell infiltration with predominance of eosinophils. Decreased mast cell numbers in the mucosa of the colon of SLMs of curcumin and pure curcumin-treated rats was observed.

Conclusions The degree of colitis caused by administration of DSS was significantly attenuated by colonic delivery of SLMs of curcumin. Being a nontoxic natural dietary product, curcumin could be useful in the therapeutic strategy for inflammatory bowel disease patients.

Keywords angiogenesis; colonic delivery; curcumin; inflammatory bowel disease; solid lipid microparticles (SLMs)

Introduction

Inflammatory bowel disease (IBD), such as ulcerative colitis and Crohn's disease, is associated with chronic relapsing inflammation of the intestinal tract of unknown aetiology.[1] Histologically, mucosal accumulation of leucocytes is a characteristic feature of IBD and activation of T cells and monocytes/macrophages has been regarded as a crucial factor in its pathogenesis. Treatment of IBD depends on drugs such as 5-aminosalicyclic acid, corticosteroids, azathioprine, mercaptopurines and ciclosporin.[1,2] However, use of these

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drugs is sometimes limited by drug-induced toxicity. There is increasing need for alternative agents that may be equally, or more, effective but toxicity free, as well as less expensive.

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), a low-molecular-weight polyphenol derived from the rhizomes of turmeric (*Curcuma longa* Linn.), is a yellow pigment, widely used as a coloring agent and spice in many foods and has been used in Ayurvedic and Chinese medicine for centuries. Turmeric contains bioactive substances known as curcuminoids, such as curcumin, demethoxycurcumin, bisdemethoxycurcumin and dimethoxycurcumin.[3] Interest in this dietary polyphenol has grown in recent years due to its vast array of beneficial pharmacological effects, including antioxidant, anti-inflammatory, anticarcinogenic,[4–6] hypocholesterolaemic,[7] antibacterial,[8] wound healing, antispasmodic, anticoagulant, antitumour[9] and hepatoprotective[10] activity. It has also been reported that curcumin has a protective effect on 2,4,6-trinitrobenzene sulfonic acid-induced colitis in mice; curcumin reduced significantly the degree of both neutrophil infiltration and lipid peroxidation, as well serine protease activity.[11] It is also a potent free radical scavenger, having superoxide anion, singlet oxygen, hydroxyl radical scavenging and lipid peroxidation inhibitory activity.[12]

Despite the promising biological effects of curcumin, its poor oral bioavailability in both rodents and humans, as reported by several workers,[13,14] has restricted its use in the management of human ailments. The main drawbacks for the clinical application of curcumin are its low solubility in water at acidic and physiological pH[9] and its rapid hydrolysis under alkaline conditions.[15,16] Extensive presystemic metabolism of curcumin may be responsible for the unfavourable pharmacokinetics of this molecule. These problems can be addressed by incorporation of curcumin into a colloidal carrier system and other methods, such as administration of curcumin along with piperine, use of liposomal curcumin, nanoparticles of curcumin and synthetic analogues of curcumin. Among modern drug delivery carriers solid lipid microparticles (SLMs) seem to be a promising colloidal carrier system. SLMs made from biodegradable solid lipids exist in the submicron size range and can be prepared by several methods. The advantages of SLMs are as follows: the possibility of controlled drug release and drug targeting; protection of incorporated compound against chemical degradation; no biotoxicity of the carrier; avoidance of organic solvent; controlled release characteristics; chemical and physical storage stability (for both drug and carrier) and feasibility of scaling up production.[17–19]

Angiogenesis is a strictly controlled process in the normal human body and is regulated by a variety of endogenous angiogenic and angiostatic factors.[20] However, at short notice the microvascular system appears capable of responding with rapid capillary growth to physiological demands such as ovulation, as well as to pathologic conditions such as wounds, chronic inflammation, certain immune reactions and tumours.

In this study we have developed a novel formulation approach for treating experimental colitis in a rat model. We have prepared SLMs of curcumin with various lipids, such as palmitic acid, stearic acid and soya lecithin, with an optimized percentage of poloxamer 188 and evaluated them for increasing the solubility of curcumin. The effect on angiogenesis was determined by the chick embryo chorioallantoic membrane (CAM) system. Further, a modified pulsatile capsule has been designed to deliver the drug to the terminal ileum and colon. Modified pulsatile capsules were designed by filling the optimized SLM formulation of curcumin into a hard gelatin capsule followed by coating the capsule with acrylic polymers (pH-dependent polymers). The developed delivery system was evaluated in a dextran sulfate (DSS)-induced experimental colitis model for its potential as a new therapeutic approach for treatment of IBD.

Materials and Methods

Materials

Curcumin was a gift from Natural Remedies Pvt Ltd (Bangalore, India). Soya lecithin, stearic acid and palmitic acid were purchased from Hi-Media (Mumbai, India). Methanol and Tween-80 were purchased from Thomas Baker (Mumbai, India). Poloxamer 188 was a gift from BPRL (Bangalore, India). Dialysis membrane, diameter 76 mm, with a number 150 was purchased from Hi-Media (Mumbai, India). Eudragit S 100, Eudragit L 100, Eudragit RL 100 and Eudragit RS 100 were obtained from Rohm GmbH (Degussa India Pvt. Ltd, Mumbai, India). Empty porcine hard gelatin capsule No. 09 was obtained as a gift sample from TORPAC Inc. (NJ, US). All other chemicals used were of analytical grade.

Preparation of curcumin loaded solid lipid microparticles

Curcumin loaded SLMs were prepared by a modified microemulsion technique according to the method developed by Gasco.[21] The oil phase, consisting of 1–10% (w/w) lipid and 1% (w/w) curcumin, was heated to 45°C in a china dish. The aqueous phase, consisting of 0.5% (w/w) poloxamer 188 and de-ionized water, was heated to 35°C. The melted mass of drug and lipid was added drop-wise and stirred at 8000*g* (Remi Sales, Bangalore, India). The microemulsion obtained was sonicated using a Probesonicator (12T-probe; Roop TELSONIC Ultrasonix Ltd, Mumbai, India) for 5 min and immediately cooled to 8°C in a refrigerator for one week. Due to the small particle size and the presence of emulsifiers, lipid crystallization may be highly retarded and the sample may remain as a supercooled melt for several months.[22]

Initial characterization of solid lipid microparticles of curcumin

The samples of pure curcumin and SLMs of curcumin were subjected to initial characterization immediately after their preparation as follows.

Vesicle size and size distribution

SLM formulations of curcumin were centrifuged at 14 000*g* at 10°C for 40 min. The supernatant was removed and the residue was washed twice with distilled water and further centrifuged at the same speed for 30 min. The residue was redispersed in water, and the mean particle size and size

Table 1 Drug content, entrapment efficiency and particle size analysis of solid lipid microparticles

No.	Code	Ratio	Lipid used	Drug content (%)	Entrapment efficiency (%)	Size of SLMs (μm)
1	F1	1 : 1	Soya lecithin	87.45 ± 1.22	68.12 ± 0.12	290 ± 2.12
2	F2	1 : 2	Soya lecithin	82.74 ± 1.11	62.35 ± 0.06	315 ± 1.66
3	F3	1 : 3	Soya lecithin	76.45 ± 1.66	58.4 ± 0.18	342 ± 1.78
4	F4	1 : 1	Stearic acid	94.67 ± 1.22	79.24 ± 0.21	108 ± 0.25
5	F5	1 : 2	Stearic acid	91.34 ± 1.18	73.83 ± 0.28	268 ± 1.23
6	F6	1 : 3	Stearic acid	84.56 ± 1.02	67.29 ± 0.11	298 ± 1.66
7	F7	1 : 1	Palmitic acid	92.93 ± 1.11	74.58 ± 0.03	131 ± 2.22
8	F8	1 : 2	Palmitic acid	87.35 ± 1.33	72.56 ± 0.06	275 ± 1.44
9	F9	1 : 3	Palmitic acid	83.26 ± 1.66	66.29 ± 0.11	312 ± 1.54

Vales are means ± SD, $n = 6$. SLMs, solid lipid microparticles.

distribution were determined using a Malvern Mastersizer (Hydro 2000 S units). The sample was placed in an automated dispersion unit and subjected to particle size analysis (Table 1). A collimated laser beam was made incident to the suspended sample particles. The intensity signals of the scattered light were processed into particle size distribution.

Drug content

The amount of drug present in a 10-mg equivalent amount of SLMs was determined by dissolving the SLM in Triton X-100 (0.1% in methanol) and the dispersion was centrifuged at 14 000*g* for 30 min. Further, it was suitably diluted with methanol and the visible spectrum was measured at 425 nm. Drug concentration was determined spectrophotometrically from a standard graph of drug developed in methanol using a Shimadzu-1700 UV-Visible spectrophotometer. Dummy SLMs of the same lipid were used as the blank. Results are shown in Table 1.

Drug entrapment efficiency

All formulations were centrifuged at 14 000*g* at 10°C for 40 min. The supernatant was removed and the residue was washed twice with water and further centrifuged at the same speed for 30 min. The residue was redispersed with Triton X-100 (0.1% in methanol) solution to obtain a clear solution. Further, dilutions were made with methanol and the absorbance was measured at 425 nm. Blank was prepared by using dummy SLMs of the same lipid and the entrapment efficiency (shown in Table 1) was calculated as follows.

$$\% \text{ Entrapment efficiency} = (\text{Drug present in SLM}/\text{Total drug content}) \times 100 \quad (1)$$

Lyophilization

Microemulsions containing SLMs of curcumin were freeze dried using a 1 : 1 ratio (on weight basis) of sucrose and lipid. Non-encapsulated drug was removed by centrifugation at 14 000*g* at 10°C for 40 min. The supernatant was removed and the residue was washed twice with water and further centrifuged at the same speed for 30 min. The dispersion was frozen to −35°C and freeze drying was performed in a freeze drier (Operon, Japan) until a dry powder was obtained. The whole drying process took 24 h with primary drying at −40°C for 16 h and secondary drying at −20°C for 8 h.

In-vitro release study

In-vitro drug release studies were performed for pure curcumin and the SLMs of curcumin to select the appropriate formulation for further analysis. In-vitro drug release from the formulations was determined by using dialysis bags No. 150 (Hi-Media, Mumbai, India). SLMs of curcumin equivalent to 25 mg pure curcumin was transferred to a dialysis bag. The bag was suspended in a 250-ml beaker containing 100 ml simulated intestinal solution consisting of a pH 6.8 phosphate buffer and PEG 400. The beakers were placed on a magnetic stirrer (Remi, Bangalore, India), the temperature was maintained at 37 ± 1°C throughout the experiment, and rotated at 50*g*. Samples of dissolution medium were collected periodically and replaced with a fresh dissolution medium. A sample of 5 ml was withdrawn and filtered through Whatman filter paper No. 1 and 1 ml of the filtrate was made up to 10 ml with methanol in a 10-ml volumetric flask. Suitable dilutions were further made when required. The samples were analysed by a spectophotometric method. Cumulative percentage drug release was calculated using PCP Disso Version 2.08 software (Poona College of Pharmacy, Pune, India); the time required for 50% and 85% drug release was calculated based on the Korsmeyer and Peppas model.[23] Empty SLMs without the drug were also subjected to an in-vitro release study. The sample obtained served as a blank for analysis. In-vitro release studies were carried out for pure curcumin.

Kinetic modelling of drug release

The dissolution profile of all the batches was fitted to zero-order, first-order, Higuchi, Hixon-Crowell, Korsmeyer and Peppas and Weibull models to ascertain the kinetic modelling of drug release by using PCP Disso Version 2.08 software, and the model with the highest correlation coefficient was considered to be the best model.

Infrared studies

The instrument used was a Shimadzu FTIR-8700 spectrophotometer. In this study, the potassium bromide disc method was employed. Both pure drug and SLM formulations were subjected to infrared (IR) studies. A pinch of powdered SLM sample was well mixed with 20 mg of dry

powdered potassium bromide. The mixture was then compressed into a transparent disc under high pressure.[24] The disc was placed in an IR spectrophotometer using a sample holder and the spectrum was scanned over a wavenumber range of 4000–400 cm^{-1}.

Scanning electron microscope studies

The surface morphology of the layered sample was examined by using a scanning electron microscope (SEM) (Akashi SX-40, Japan). A small amount of powder was manually dispersed onto a carbon tab (double adhesive carbon coated tape) adhered to an aluminium stub. These sample stubs were coated with a thin layer (30 Å) of gold by employing a Polaron-E 3000 sputter coater. The samples were examined by SEM and photographed under various magnifications with direct data capture of the images onto a computer.

Differential scanning calorimetry studies

Differential scanning calorimetry (DSC) studies of pure drug and all the selected SLM formulation was performed by using differential scanning calorimeter (Mettler Toledo Star system). Samples were weighed (5.00–8.00 mg ± 0.5 mg) and placed in sealed aluminium pans. The coolant was liquid nitrogen. The samples were scanned at 10°C/min from 20°C to 300°C. DSC thermograms of pure drug and SLMs were recorded.

X-ray diffraction studies

X-ray diffraction patterns of the selected SLM formulation was determined using a diffractometer (SMART & SAINT, Bruker, 2000) equipped with a rotating target X-ray tube and a wide-angle goniometer. The X-ray source was Kα radiation from a copper target with graphite monochromator. The X-ray tube was operated at a potential of 50 kV and a current of 150 mA. The range (2θ) of scans was from 0 to 70° and the scan speed was 2°/min at increments of 0.02°, respectively.

Filling and capsule coating for colon specific delivery

Selected SLM formulations of curcumin based on particle size, drug entrapment, drug content and in-vitro release were chosen for colon-specific delivery by developing pulsatile capsules. Around 80 mg of formulation was filled into capsules manually. Capsules were coated with Eudragit RS100, RL100, S100 and L100 and were soaked for 20 min in acetone containing the specified quantities of talc and PEG 6000. The various parameters of coating conditions were standardized, such as dipping time (5 s), coating solution concentration, temperature (25 ± 1°C), drying time (5 min) in 50°C air. The coating process was carried out until a desired weight was gained. The capsules were coated with two coats, the first was with pH-independent polymer (Eudragit RS100 and Eudragit RL100 in a ratio of 6 : 2) and the second with pH-dependent polymer (Eudragit S100 and Eudragit L100 in a ratio of 60 : 40). PEG 6000 was used as plasticizer in both the coatings. Capsules were placed in between the arms of the forceps and dipped into the coating solution for 5 s and dried artificially for 5 min. This alternate dipping and drying was repeated until the desired weight was gained by the capsule. The filled capsules were subjected to further study for site-specific delivery of curcumin in the terminal ileum.

Angiostatic activity in the chick embryo chorioallantoic membrane

The angiostatic activity of the selected SLM formulation of curcumin was demonstrated using chick chorioallantoic membrane (CAM). Fertilized, domestic five-day-old chick embryos were purchased from a poultry farm (GKVK Veterinary Sciences, Hebbal, Bangalore, India) and were incubated at 37 ± 1°C under 60% relative humidity. A small (0.5–0.6 cm^2) opening was made with a 4.0-mm scalpel blade, through the shell at the end of the egg directly over the embryonic blood vessels, as previously determined by candling, to observe the growth, development and location of the embryo. The shell membrane was removed slowly and carefully. Selected SLMs of curcumin weighing about 15 ± 0.2 mg containing curcumin (5 mg) and pure curcumin 5 mg/ml (ethanol–PBS 1 : 9 v/v), respectively, were transferred directly on to the CAM surface and the shell was closed using parafilm. After 48 h of incubation the vasculature was observed using a dissecting microscope at a magnification of ×10 and photographs were taken using a 4.2 mega pixel camera (Cannon cybershot Model V3X). Six eggs were tested in each group and the experiment was performed in triplicate to ensure reproducibility.

In-vivo study

All experimental procedures were in accordance with guidelines for animal welfare set out by the Medical Research Council of India. Animal Ethics Committee approval was obtained. Male Sprague–Dawley rats, aged 6–7 weeks (175–205 g), obtained from the Indian Institute of Science (Bangalore, India), were used for in-vivo evaluation. They all received the standard laboratory diet and were not fasted before the experiment; they were allowed free access to drinking water except during the induction of colitis. Rats were housed (two per cage) and kept in a regulated environment (temperature, 22 ± 1°C; humidity, 50 ± 5%; light–dark cycle, 12 h). The rats were randomized into four groups ($n = 6$). Rats in groups 1, 2 and 3 received 5% dextran sulfate sodium (DSS) solution (MW 40 000; Sigma Chemicals, Gujarat, India) in drinking water for 9 days. Control rats received plain drinking water. After nine days, rats in group 1 received SLMs of curcumin, 100 mg/kg (body weight) in pulsatile capsules,[25] rats in group 2 received pure curcumin (100 mg/kg in pulsatile capsules) and rats in group 3 served as a DSS control. The clinical results recorded in DSS-treated and control rats were body weight, stool consistency, faecal bleeding, and diarrhoea.

The experiment was terminated at the end of 90 days and all rats were killed by cervical dislocation after overnight fasting. The colon was slit open longitudinally and cleaned of faecal material with a gentle jet of water. Then the colon was stored in formalin (10%) solution and subjected to histopathological examination.

Assessment of inflammation: symptoms and inflammatory score

Clinical assessment of inflammation included weekly monitoring of body weight and general health. The macroscopic appearance of the colon (inflammatory score), based on the degree of inflammation and the presence of oedema or ulceration, stool consistency (diarrhoea score) and visible faecal blood, were scored separately on a scale of 0–3.[26]

The distal colon, proximal colon and ileum were studied. Each specimen was sliced down the antimesenteric border to give two symmetrical halves, stretched and fixed in 10% buffered formalin for 4 h and then rinsed in 70% alcohol. The formalin-fixed sections were embedded in paraffin blocks and sections were cut, dewaxed and stained with haematoxylin–eosin. The severity of inflammation was assessed first macroscopically. The freshly opened colon was examined under a magnifying glass by two independent observers blinded to the procedure. The extent of mucosal damage was assessed using the macroscopic inflammatory scoring (MIS) system.[27]

Histology

Tissue sections (5 μm) of distal and proximal colon were stained with haematoxylin–eosin to address the degree of inflammation and with Masson's trichrome to visualize the connective tissue. The stained tissue was analysed independently and in a blinded fashion with a standard microscope (Nikon Charge couple device (CCD) with digital display at ×40 and ×100 magnification). The pathophysiology of the tissue was characterized and the lesions were scored as described by Vilaseca *et al*.[26] By the presence of ulceration, inflammatory cells (neutrophils, macrophages, lymphocytes and plasma cells), signs of oedema, crypt loss, surface epithelial cell hyperplasia, goblet cell reduction and signs of epithelial regeneration were examined.[28]

Statistical analysis

All values are expressed as the mean ± SD. Statistical analysis was performed using analysis of variance for dependent variables. $P < 0.05$ was considered significant.

Results

Curcumin-loaded SLMs were prepared successfully by a microemulsion technique at a temperature range of 35–40°C. An oil-in-water microemulsion was spontaneously obtained as recognized by a clear solution after adding the oil phase into the heated water phase of the same temperature. The SLMs were obtained immediately when dispersing the melted lipid into warm water with the aid of high-speed searing. The microemulsion obtained was sonicated for 5 min and immediately cooled to 8°C in a refrigerator for one week.

Mean particle size and size distribution

The mean particle size and size distribution of the curcumin-loaded SLMs were determined. The results showed that the amount of lipid and emulsifier (poloxamer 188) were critical parameters for the size of the microparticles. The effect of soya lecithin, stearic acid and palmitic acid concentration on the mean particle size was evaluated by varying the concentration (1%, 5% and 10% (w/w)), while maintaining the amount of poloxamer at 0.5% (w/w). When the lipid concentration was increased from 1% to 10% (w/w), the size distribution with the mean particle size increased from 108 ± 0.25 to 342 ± 1.78 μm (Table 1). This finding was in agreement with Mehnert and Mader,[18] who reported that increasing the lipid content over 1–10% (w/w) in most cases resulted in larger mean particle sizes and broader size distributions. Therefore, 1% (w/w) stearic acid was found to be an optimum concentration for the formulation of the SLMs.

Determination of drug content and incorporation efficacy

Methanol was used to break the SLMs and encapsulated drug was obtained for analysis. The formulations studied demonstrated moderate-to-high curcumin incorporation efficacy in the range of 58.40 ± 0.18% to 79.24 ± 0.21% (w/w). All SLM formulations had a percentage drug content of 76.45 ± 1.66 to 94.67 ± 1.22 (Table 1).

In-vitro release of pure curcumin and solid lipid microparticle formulations

Curcumin possesses very poor aqueous solubility. Thus, to provide sink conditions 10% (v/v) PEG 400 in simulated intestinal fluid was chosen as the receptor medium. The release potential of curcumin from the lipid particles was evaluated over 24 h. Each sample was analysed in triplicate using a spectrophotometric method. The in-vitro release studies from pure curcumin and SLMs demonstrated prolonged release characteristics (Figure 1). The rate of dissolution was found to be increased in SLMs as shown by the time taken for 20 and 50% of drug to be released. Formula F3 showed a burst release pattern initially and more than 50% of the drug was released in 12 h, while for pure curcumin more than 24 h was required for 50% of curcumin

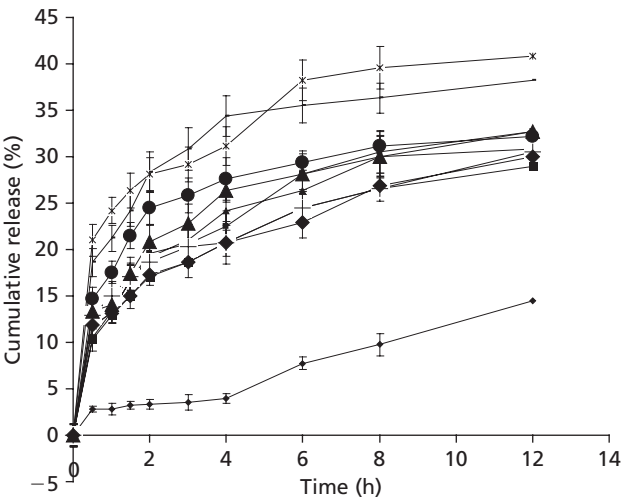

Figure 1 Effect of polymers and surfactant concentrations on release profile of curcumin. ◆, pure drug; ■, F1; ▲, F2; ■, F3; ×, F4; ●, F5; +, F6; –, F7; ▲, F8; ◆, F9. Values are means ± SD, $n = 6$.

Table 2 In-vitro dissolution data of solid lipid microparticle formulations of curcumin

No.	Formulation	T_{20} (time for 20% drug release)	T_{50} (time for 50% drug release)
1	Pure drug	12.0 ± 0.32	>24
2	F1	2.33 ± 0.48	21.1 ± 2.9
3	F2	3.27 ± 0.72	22.5 ± 2.5
4	F3	4.11 ± 0.63	23.8 ± 2.7
5	F4	0.51 ± 0.12	12.2 ± 1.2
6	F5	1.36 ± 0.23	14.3 ± 1.4
7	F6	3.12 ± 0.98	17.8 ± 1.7
8	F7	0.78 ± 0.19	13.9 ± 1.4
9	F8	1.98 ± 0.45	15.7 ± 2.2
10	F9	3.87 ± 0.99	16.2 ± 2.8

Values are means ± SD, $n = 6$.

to be dissolved. The concentrations of various lipids were further increased to obtain the desired release profile but the other formulations showed no satisfactory drug release. The effect of the lipid concentration on the release profile of curcumin is shown in Figure 1 and Table 2. SLM formulation F4 had the smallest particle size, 108 nm, compared with other formulations (Table 1). The stearic acid formulation had enhanced release in the dissolution media when compared with pure curcumin and other formulations. This formulation was therefore selected for further evaluation.

Kinetic modelling of drug release

Linear regression analysis and model fitting showed that all the formulations followed the Korsmeyer and Peppas model, which had the highest value of correlation coefficient, *r*. Thus, the release of curcumin was controlled by the Korsmeyer and Peppas dissolution model:

$$\log \%R = \log K + n \log t \quad (2)$$

where %R is the percentage drug release at time t, K is a release rate constant and n is the diffusional release exponent that could be used to characterize the different release mechanisms ($n = 0.5$ (Fickian diffusion), $0.5 < n < 1$ (anomalous transport), $n = 1$ (case II transport; i.e. zero-order release) and $n > 1$ (super case II transport)). This model is widely used when the release mechanism is not well known or when more than one type of release phenomenon could be involved.

Lyophilization

Lyophilization has been adopted to increase the stability of the formulated SLMs. The freeze drying cycle was optimized for efficiency. In this study, sucrose was chosen as a lyoprotectant. Use of sucrose with lipid gave a free-flowing powder.

Infrared studies

The FT-IR spectra of curcumin and stearic acid were determined. The prominent peaks in curcumin are as follows: (1) 3595 cm^{-1} to phenolic (OH) vibrations; (2) 3075 cm^{-1} to aromatic C–H stretching vibrations; (3) 1600 cm^{-1} to the stretching vibration of benzene ring skeleton; (4) 1510 cm^{-1} to the mixed (C=O) and (C=C) vibration; (5) 1425 cm^{-1} to the olefinic C–H in-plane bending vibration (δ_{C-H}); (6) 1280 cm^{-1} to the Ar–O stretching vibration. In the FT-IR spectra of the physical mixtures there were no changes. The broad absorption bands at 3410 cm^{-1} arose from the stretching mode of OH groups. The bands in the range of 3079–3000 cm^{-1} could be attributed to aromatic C–H stretching vibration, which may indicate intercalation of curcumin in lipid.

Scanning electron microscope studies

Figure 2 shows SEM photographs of pure curcumin, drug-free SLMs, a physical mixture and SLMs containing 1 : 1 curcumin–lipid at different magnifications. Stearic acid (blank) microparticles were spherical with varying sizes, smooth surface and individually homogeneously distributed without apparent porosity. The crystals of pure curcumin exhibited an irregular shape. In the physical mixture, the characteristic curcumin particle, which was mixed with lipid, adhered on the surface of the lipid. SLMs had a spherical shape and a smooth surface. Moreover, drug crystals were observed on the surface of the microparticles. SEM photographs revealed a compact structure without cavities of internal porosity. However, particles were slightly aggregated in nature and the original morphology of both the components had disappeared.

Thermal characterization of solid lipid microparticles of stearic acid containing curcumin

DSC thermograms were performed for pure curcumin, stearic acid, a physical mixture and SLMs of curcumin. To identify the physical state of the drug within the microparticles, selected formulations were subjected to DSC studies. A sharp and large melting onset peak/endset peak of curcumin at 160.86/180.00/193.14°C was obtained. The physical mixture of lipid and curcumin showed persistence of the endothermic peak of both the constituents. Here, the drug endothermic peak shifted to 119.32°C showing reduced intensity; this may have been due to the presence of stearic acid. However, the melting peak of the drug was absent in the DSC thermogram of stearic acid SLMs containing curcumin, indicating that the drug may be uniformly dispersed in the microparticles as an amorphous form. This phenomenon may be indicative of a strong interaction between curcumin and stearic acid in the complex.

X-ray diffraction

X-ray diffraction patterns were performed for pure curcumin, stearic acid, SLMs (1 : 1, drug–lipid) and physical mixture. Curcumin showed the characteristic peaks, which were displayed in the wide-angle regions pointing to the crystalline nature of curcumin particles. As expected, these polymorphic forms were not visible in the X-ray pattern of SLMs of curcumin, as already confirmed in the thermal event (melting endotherm) in the DSC heating runs. X-ray

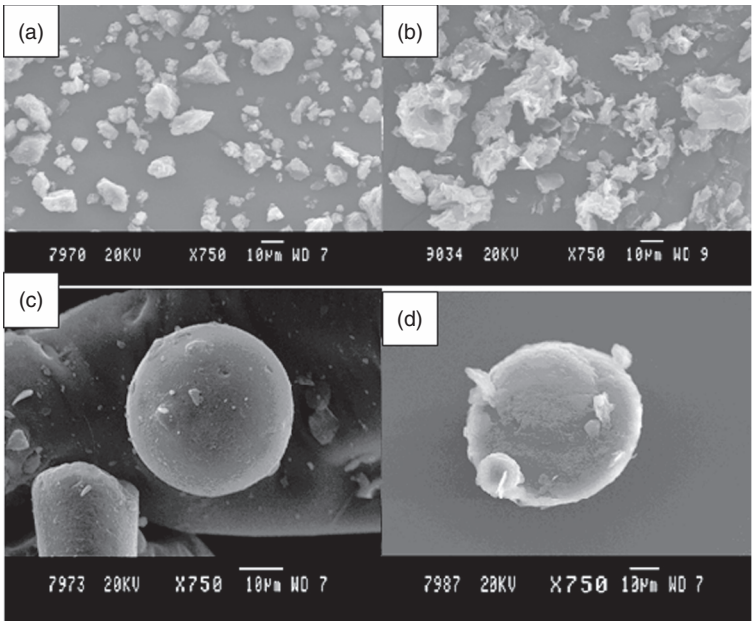

Figure 2 Scanning electron microscope of curcumin and its solid lipid microparticles (SEMs). (a) Pure curcumin; (b) physical mixture; (c) blank SLMs of stearic acid; and (d) SLMs of curcumin.

diffraction data only allow differentiation between crystalline data and amorphous material.

Angiostatic activity in the chick embryo chorioallantoic membrane study

The CAM study was carried out to comparatively evaluate the anti-angiogenic activity of curcumin and SLMs of curcumin. Figure 3 shows CAM with control (a, b), which showed a normal capillary network structure. The CAM treated with curcumin (c, d) and SLMs of curcumin (e, f) showed vascular regression and zones which were devoid of a capillary network and which clearly indicated the angiostatic activity of SLMs of curcumin and pure curcumin.

Assessment of inflammation in rats: symptoms and inflammatory score

Oral administration of DSS in drinking water resulted in extensive haemorrhagic and ulcerative damage to the distal colon as observed up to 90 days. Macroscopic examination of the distal colon and rectum from DSS-treated rats revealed the presence of multiple mucosal erosions and ulceration. The colon and rectum showed evidence of mucosal congestion, erosions and haemorrhagic ulceration evaluated at 10, 15 and 25 days as assessed by damage score.

Treatment of rats with curcumin and SLMs of curcumin resulted in a significant decrease in the extent and severity of the injury of the large intestine as demonstrated by disease activity index (Figure 4) as well as histopathological assessment (Figure 5).[26] The observed inflammatory changes in the large intestine were associated with an increase in weight of the colon as well as a significant increase in body weight as compared with DSS control rats (Figure 4a). In contrast, no significant increase in weight was found for the colon of DSS-treated rats. Colon weight/length ratio, a marker of tissue oedema, was significantly higher in DSS-treated rats than in rats treated with curcumin or SLMs of curcumin ($P < 0.05$). Moreover, treatment with curcumin and its SLMs also significantly reduced the loss in body weight, which correlated well with the recovery of the colonic injury. During the 90-day period, rats receiving SLMs of curcumin showed a greater level of reduction in disease activity index than those receiving pure curcumin.

Histology

No obvious macroscopic inflammation was observed at the colon surface of rats treated with curcumin and SLMs of curcumin (Figure 5). A mild microscopic increase in chronic inflammation was observed in DSS control rats (Figure 5b). At day 90, rats treated with SLMs of curcumin showed minimal changes in the surface epithelium; no infiltration of inflammatory cells to the mucosa was observed (Figure 5c). The changes were more pronounced in both DSS control (Figure 5b) and pure curcumin control (Figure 5d), with loss of crypts and reduction of goblet cells, signs of surface epithelial regeneration and focal ulcerations. The number of neutrophils was slightly increased and remained elevated during the inflammation in the DSS and pure curcumin groups. We also found decreased eosinophils and mast cell numbers in the mucosa of the proximal colon of rats treated with SLMs of curcumin and pure curcumin.

Discussion

During the preparation of SLMs of curcumin it was observed that there was spontaneous formation of an oil-in-water microemulsion when the lipid phase containing curcumin was added to heated water, which was further size reduced by

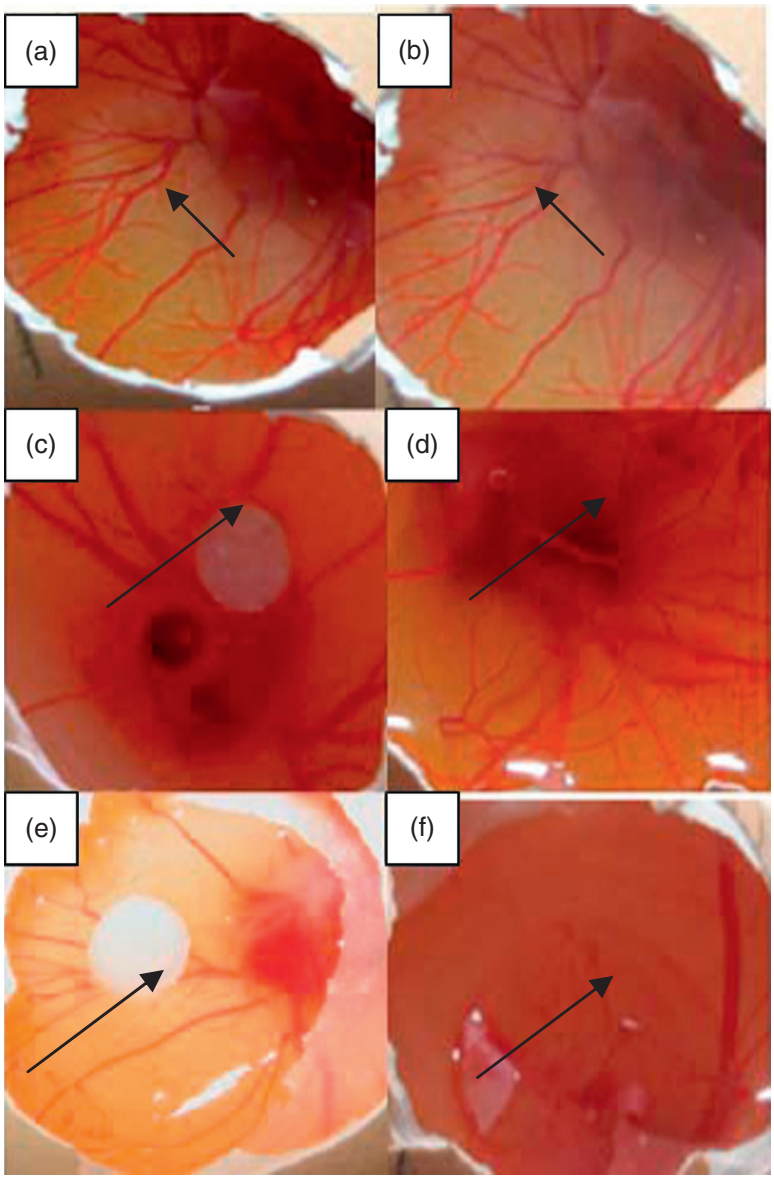

Figure 3 Antiangiogenic activity of solid lipid microparticles (SLMs) of curcumin. Representative photographs of chorioallantoic membrane: (a) visualization of vascularity and embryo in control at 0 h; (b) control after 48 h; (c) effect of pure curcumin at 0 h; (d) effect of curcumin after 48 h; (e) effect of SLMs of curcumin at 0 h; (f) effect of SLMs of curcumin after 48 h.

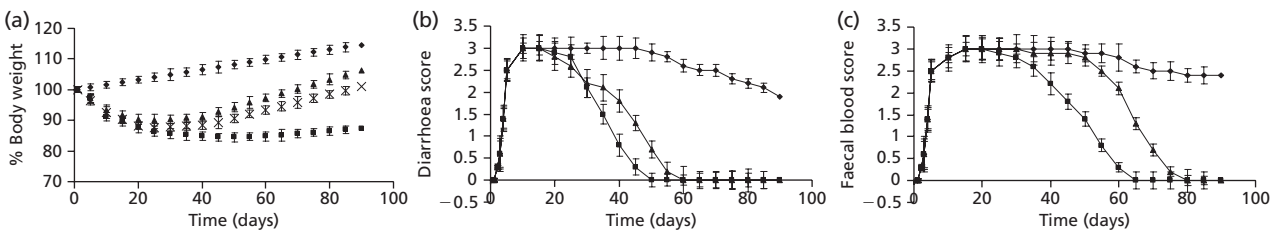

Figure 4 Effect of curcumin and its solid lipid microparticle (SLM) formulations on inflammatory markers during colitis. (a) Effect on body weight. Body weight change was calculated by dividing body weight on specified day by body weight at day 0 (starting body weight) and expressed as a percentage. ♦, control; ■, dextran sulfate solution (DSS) control; ▲, SLM; ×, pure curcumin. (b) Diarrhoea score by necropsy. (c) Visible faecal blood score by necropsy. ♦, DSS control; ■, SLM; ▲, pure curcumin. Values are means ± SD, $n = 6$.

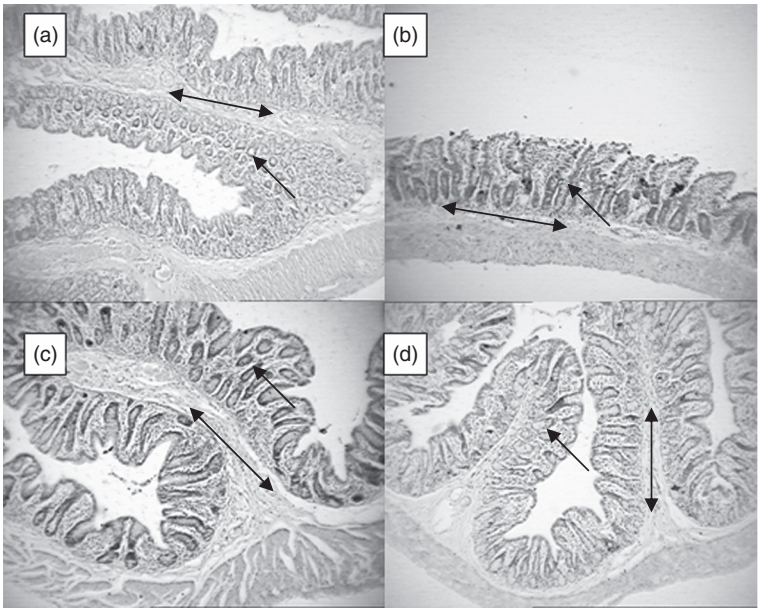

Figure 5 Effect of curcumin and its solid lipid microparticles (SLMs) on colon injury. (a) Colonic mucosa of control rat did not show any histological modifications. (b) Dextran sulfate solution (DSS)-induced mucosal injury associated with transmucosal necrosis, oedema and submucosal infiltration of inflammatory cells. (c) Effect of SLMs of curcumin showing normalization of disturbances in morphology associated with DSS treatment. (d) Effect of pure curcumin showing normalization of disturbances in morphology associated with DSS treatment. Single arrow, inter glandular space; double-sided arrow indicates mucosal thickness and cell infiltration.

high-speed shearing and sonication. In the SLMs, due to the small particle size and the presence of emulsifiers, lipid crystallization may be highly retarded and the sample may remain as a super-cooled melt for several months.[22] A drug-enriched core will be found when the drug precipitates first before the lipid recrystallizes. This could be obtained while dissolving curcumin in the lipid-melt at, or close to, its saturation solubility.[18] Cooling of the microemulsion will lead to a supersaturation of drug in the melted lipid and subsequently to drug crystallization preceding lipid crystallization. Further cooling will finally lead to the recrystallization of the lipid surrounding the drug core as a membrane. This lipid membrane will contain only drug in such a concentration corresponding to the saturation solubility of the drug at the recrystallization temperature of the lipid. This results in a drug-enriched core surrounded by a lipid shell. Further cooling will facilitate rapid lipid crystallization and prevent lipid aggregation.[18]

The drug content and incorporation efficiency results indicate that the concentration of lipid and emulsifier has critical effects on the curcumin incorporation efficacy, as shown in Table 1. Curcumin is poorly soluble in water and in lipids. It is soluble in alkaline medium and methanol. Its solubility in the aqueous phase, however, could be enhanced by the addition of surfactant. The effect of amount of lipid on the entrapment efficacy was studied by maintaining the amount of surfactant while varying the amount of lipid. Our results showed that the entrapment efficacy decreased as the amount of lipid increased due to the insolubility of the curcumin in lipid. Thus, the curcumin is incorporated in the surfactant layer at the surface of the SLMs leading to a high entrapment efficacy. On the other hand, at high lipid concentration, more SLMs were produced with insufficient amounts of surfactants to solubilize the total amount of curcumin at the particle surface resulting in low entrapment efficacy. It was found experimentally that the incorporation efficacy was optimal at 1% lipid and 0.5% (w/w) poloxamer 188, where the release of the curcumin in the aqueous phase was optimum. Therefore, increased curcumin could be released from the SLMs into micelles formed in the aqueous phase by the incorporated surfactant, resulting in increased dissolution.[29,30]

When the SLMs of curcumin were evaluated by in-vitro dissolution study an improvement in the dissolution rate of curcumin was observed. This improvement obtained with SLMs could also be due to drug wettability because of surfactant concentration. This may indicate that most of the curcumin was incorporated at the surface of the SLMs and diffused into the dissolution media until a steady state was reached.[29,30]

SLMs of curcumin were lyophilized by using sucrose as a cryoprotectant, yielding a free-flowing powder. It is well known that a cryoprotectant is necessary to decrease microparticle aggregation during the lyophilization process. In addition, the cryoprotectant could help to ease redispersion in aqueous media. Sucrose may act as a cryoprotectant by stabilizing the bilayers, especially at their phase-transition temperature, during freezing and thawing cycles. This action of sucrose is probably best explained by the particle immobilization hypothesis,[31] which suggests that a lyoprotectant protects particles against aggregation by providing spatial separation and immobilization of the particles in a glassy matrix during dehydration. Sucrose may interact with the polar head group of the surfactant molecules and thus

prevent contact between discrete particles through steric hindrance.[18,32]

DSC is a very useful tool in the investigation of thermal properties of SLMs and can provide both qualitative and quantitative information about the physicochemical state. The related thermal transitions include melting, recrystallization, decomposition and a change in heat capacity. DSC is useful for monitoring different samples of the same material to assess their similarities or differences or the effects of additives on the thermal properties of a material. Using the DSC analysis of drug, lipid and produced SLMs, the nature of the drug inside the lipid matrix can be assessed, which may emerge as in solid, metastable molecular dispersion or crystallization. However, the melting peak of the drug was absent in the DSC thermograms of stearic acid SLMs containing curcumin. This phenomenon is indicative of a strong interaction between curcumin and stearic acid in the lipid microparticles.

X-ray diffraction has been used for the study of molecular structure and polymorphism of lipid microparticles.[22,33] Therefore, these measurements were performed after 7 days of storage at room temperature to compare the crystalline nature of the investigated lipid particles. SLMs of curcumin did not contain any peaks associated with crystals of the drug, suggesting that the drug was amorphous in the SLM matrix. X-ray crystallization studies confirm that the transition rates of SLMs are lower than for pure drug. These differences may be due to the more ordered structure of SLMs of curcumin.

The term angiogenesis generally refers to the development of new blood vessels from pre-existing ones. However, in pathological conditions such as inflammatory diseases, tumour growth or tumour metastasis, a chronic 'unregulated' angiogenic state often exacerbates the disease. Thus, it has been hypothesized that there is a link between angiogenesis and inflammation. Angiogenesis inhibition is defined as a zone of avascularity, 2–6 mm in diameter, which is a direct result of occlusion, disruption, degeneration and regression. Curcumin has potent anti-angiogenic activity as demonstrated by experiments using the chick CAM assay.[25] The CAM study provides a rapid method for investigating the release of curcumin in a biological system, penetration of curcumin through the cell membrane and a comparative evaluation of the anti-angiogenic activity of the formulation. The CAM study showed vascular regression and zones that were devoid of a capillary network and which clearly indicated the angiostatic activity of SLMs of curcumin and pure curcumin.

DSS is thought to exert its action initially by affecting tight junctions with a subsequent immunological activation and alterations in luminal flora and mucus composition. A predominance of caecal injury has been noted, or distal injury in the more severe models.[34] DSS-induced colitis is characterized by histological findings such as oedema, transmucosal necrosis, infiltration of inflammatory cells into both the mucosa and the submucosa, destruction of epithelial cells and mucosal thickening. Studies have been carried to characterize the molecular events, histopathology and role of inflammation in dysplasia in a DSS model of rat colitis and draw analogy to ulcerative colitis-associated dysplasia in humans with the intent to use this model for further molecular and inflammatory research.[26] During the 90-day period, rats receiving SLMs of curcumin showed a reduced level of disease activity index compared with those receiving pure curcumin. SLMs of curcumin thus suppressed the development of DSS-induced colitis in rats.

Conclusions

Curcumin-loaded SLMs were successfully prepared by a microemulsion technique at moderate temperature. The process parameters, such as the amount of lipid and surfactant, were crucial factors for the resulting mean particle size and the efficacy of drug incorporation. At optimal conditions, the mean particle size of curcumin-loaded SLMs was 108 μm and incorporation efficacy of curcumin was 79.24% (w/w). The release kinetics of curcumin from a lipid base could be fitted with Higuchi's square-root model and showed that approximately 79.24% of curcumin was released from formulation F4 within 12 h with a 24.1% burst release within the first hour, suggesting that most of the curcumin was still incorporated in the SLMs.

Anti-angiogenic drug therapy is a promising development in anti-inflammatory and anticancer treatments. Formulation F4 exhibited anti-angiogenic properties, which was found to be greater than those of the pure drug. In in-vivo studies using a rat colitis model, the SLM formulation-treated group showed a faster weight gain than the DSS control. An additional finding in the DSS-treated rats was the predominance of eosinophils in the chronic cell infiltrate. Decreased mast cell numbers were observed in the mucosa of the colon of rats treated with the SLM formulation of curcumin and pure curcumin. This effect of curcumin may be associated with inhibition of NF-κB activation and blockade of infiltration of inflammatory cells including CD4 and CD8 T cells. This study has demonstrated that the degree of colitis caused by administration of DSS was significantly attenuated by the SLM formulation of curcumin. Being a nontoxic natural dietary product, curcumin could be useful in the therapeutic strategy for IBD patients.

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Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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