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ORIGINAL ARTICLE

Indomethacin-5-fluorouracil-methyl ester dry emulsion: a potential oral delivery system for 5-fluorouracil

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Abstract

Objective: To produce a combined effect of indomethacin (IDM) and 5-fluorouracil (5FU) for cancer therapy, the side effects of IDM on the gastrointestinal (GI) tract were reduced and the oral adsorption of 5FU was improved. Indomethacin-5-fluorouracil-methyl ester (IFM) dry emulsion was prepared and evaluated as a potential oral delivery system for 5FU. *Methods*: IFM was synthesized by formation of an ester between IDM and 5FU intermediate and then characterized by structure, melting point, solubility, apparent partition coefficient, and incubation with GI tract contents and plasma. Gum acacia and sodium carboxymethyl cellulose (CMC-Na) were applied as the adsorbent and solid carrier to prepare IFM dry emulsion. IFM dry emulsion was then characterized by reconstitution in water and in situ intestinal perfusion experiment. *Results*: Physicochemical properties of the new synthesized compound confirmed the formation of IFM. Incubation of IFM in the contents of the GI tract and plasma revealed that IFM was not relatively stable in GI contents during the time period of transit through the GI tract, whereas it was very unstable in plasma and released 5FU rapidly. The IFM dry emulsion could be easily reconstituted in water, and the mean particle size was 2.416 μm. The absorption rate constant (*K*) for IFM with concentration of 2, 5, and 10 μg/mL in the in situ perfusion experiment were 0.473, 0.423, and 0.433/h, respectively, demonstrating passive diffusion of IFM across the biological membranes. *Conclusion*: This study indicates that the IFM dry emulsion may represent a potentially useful oral delivery system for 5FU.

Key words: *5-Fluorouracil; dry emulsion; in situ perfusion experiment; indomethacin; indomethacin-5-fluorouracil-methyl ester*

Introduction

The fluoropyrimidine anticancer agent 5-fluorouracil (5FU) is a commonly used drug to treat a wide range of solid tumors, particularly gastric, colorectal, and head cancers. Until now, 5FU has been the only chemotherapeutic agent with significant activity for the treatment of colorectal cancers[1]. However, 5FU is usually administrated as an intravenous bolus or continuous infusion, resulting in gastrointestinal (GI) side effects, and is inconvenient for patients. Oral administration of 5FU alone demonstrates erratic and decreased absorption and nonlinear pharmacokinetics, because of the high activity of dihydropyrimidine dehydrogenase (DPD), a catabolizing enzyme of 5FU, which causes rapid metabolism of 5FU[2–5]. This problem may be overcome by the oral coadministration of drugs that inhibit 5FU degradation in the GI tract. Alternatively, other fluoropyrimidine derivatives may be administered, which are absorbed intact and subsequently converted to 5FU. Reports on the development of 5FU prodrugs (i.e., tegafur, doxifluridine, or capecitabine) have appeared aiming at enhancing its oral absorption and reducing its first-pass metabolism[5,6]. The oral agents that have recently been evaluated in clinical trials include capecitabine, S1, and uracil-ftorafur (UFT)[7].

Indomethacin (IDM), a nonsteroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties, has been widely used to reduce inflammation and pain in patients. Interestingly, substantial experimental and

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clinical evidence indicates a role for NSAIDs in the prevention of various types of cancer, especially when combined with chemotherapy[8–10]. Kapitanović et al. presented the effect of IDM on growth inhibition, induction of apoptosis, and alterations in the expression of several genes involved in Wnt signaling in HT-29 colon cancer cells. Fujino et al. demonstrated that treatment of LS174T cells with IDM could cause a downregulation of EP2 prostanoid receptor expression that may be independent of COX inhibition[11,12]. However, their efficacies are offset by a significant incidence of GI ulceration and hemorrhage because of the presence of carboxyl group. Many attempts, such as prodrug strategies[13,14], have been made to reduce the side effects associated with NSAIDs.

Dry emulsions present a potential oral drug delivery system for lipophilic and poorly soluble drugs. They are powdery, lipid-based formulations, from which an oil-in-water (o/w) emulsion can easily be reconstituted in vivo[15]. They possess the same advantages of emulsions by improving the absorption and bioavailability of poorly water-soluble drugs and circumvent the instability problem associated with conventional emulsions[16–18].

In light of the above considerations, to produce a combined effect of IDM and FU for cancer therapy, the side effects of IDM on the GI tract were reduced, the oral adsorption of 5FU was improved, a new prodrug of 5FU was synthesized as indomethacin-5-fluorouracil-methyl ester (IFM), and furthermore, IFM dry emulsion was prepared. IFM was synthesized and characterized by determining its melting point, solubility, partition coefficients, ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometric (MS) spectra. Incubation with contents of the GI tract and plasma was carried out to evaluate the hydrolysis of IFM under physiological conditions. Furthermore, IFM dry emulsion was prepared and characterized after reconstitution. An in situ intestinal perfusion experiment was performed to estimate the absorption of IFM dry emulsion in the GI tract.

Materials and methods

Materials

5FU (99.9%) was kindly supplied by Nantong Haiers Pharmaceutical Co., Ltd. (Jiangsu Province, China). IDM (99.5%) was purchased from Shijiazhuang Pharmaceutical Group Huasheng Pharma Co., Ltd. (Hebei Province, China). *N*,*N*-Dicyclohexylcarbodiimide (LR) was obtained from Shanghai Tianlian Fine Chemical Co., Ltd. (Shanghai, China). 4-Dimethylaminopyridine (DMAP) was obtained from Jiangsu Wuxi Bisheng Chemical Co., Ltd. (Jiangsu Province, China). Sodium carboxymethyl cellulose (CMC-Na) and medium-chain triglyceride (MCT) were purchased from Shanghai Chemical Agent Co., Ltd. (Shanghai, China). Other reagents and solvents used were of analytical/spectroscopic high-performance liquid chromatography (HPLC) grade as required.

Synthesis and characterization of indomethacin-5-fluorouracil-methyl ester

Synthesis of 1,3-dimethylol-5-fluorouracil

5FU (1.30 g, 10 mmol) and formaldehyde (1.78 g, 22 mmol) solutions (37%) were added to a round bottom flask and heated on a water bath at 60–65°C until dissolved. The reaction solution was stirred for 50 minutes and concentrated under reduced pressure to remove excess water and formaldehyde. A white oily crude product was obtained as 1,3-dimethylol-5-fluorouracil.

Synthesis of indomethacin-5-fluorouracil-methyl ester

The above intermediate product was transferred to a round bottom flask, to which 150 mL anhydrous acetonitrile, IDM (4.3 g, 12 mmol), DDC (2.47 g, 14 mmol), and DMAP (0.08 g) were then added. The mixture was stirred for 72 hours at room temperature, and the progress of the reaction was monitored by thin-layer chromatography. The mixture was then filtered and evaporated under vacuum. The obtained residue was dissolved in ethyl acetate, washed with dilute hydrochloric acid, saturated sodium bicarbonate solution, and distilled water. The organic phase was dried over anhydrous sodium sulfate, filtered, and purified by column chromatography using 10% acetone/dichloromethane as the eluting system to obtain the purified conjugate. The overall actual yield was 66%.

The melting point was determined in an open capillary tube on a Sonar melting point apparatus and was uncorrected. The structures of the synthesized compounds were confirmed by spectral (UV, IR, NMR, and MS) data analysis. A 4802H double beam spectrophotometer was used for UV absorption determination of the compounds. The IR spectra were recorded using KBr disks on a Bruker Is-55 infrared spectrometer. The ^{1}H-NMR spectra were recorded on a Bruker-ARX 300 spectrometer operating at 300 MHz in dimethyl sulfoxide-d6 (DMSO-d6). Chemical shifts were reported in parts per million downfield from the internal standard, tetramethylsilane. MS were recorded on a GCMS-QP5050A analyzer.

HPLC analysis

The free FU and IFM were assayed by reversed-phase HPLC[19]. The chromatographic mobile phase consisted of methanol:water:36% acetic acid (3:96.9:0.1) and methanol:25 mM ammonium acetate (70:30) for 5FU

and IFM, respectively. The flow rates and detection wavelengths were 1.0 mL/min and 260 nm. The operating temperature was ambient and the injected volume was 20 μL. The HPLC system consisted of a HITACHI L-7100 pump, a UV–Vis L-7420 detector, and a Diamonsil C_{18} (200 × 4.6 mm, 5 μm) column. 5-Bromouracil and diclofenac sodium were used as internal standards for 5FU and IFM, respectively. The mobile phase was prepared daily, filtered, and degassed by ultrasonication before use.

Solubility studies

The solubility of IFM was determined at room temperature in a variety of solvents. An excess of IFM was equilibrated with each solvent in a screw-top vial with frequent shaking (180 rpm), vortexing, and sonicating. The saturated solution was passed through a 0.45 μm Millipore filter, and the filtrate was analyzed by HPLC after appropriate dilution with mobile phase.

Apparent partition coefficient studies

Classic shake-flask method was applied[20]. A known concentration (C_0) of IFM in 1-octanol saturated with buffer solution was vortexed for 2 hours with an equal volume of buffer solutions (pH 1.2 and 7.4, respectively) saturated with 1-octanol in a screw-top vial. The equilibrium concentrations of IFM in 1-octanol (C) were determined by HPLC and each experiment was performed in triplicate. The apparent partition coefficients were calculated using the following equation, and the values did not vary by more than 10%.

$$P_{\text{app}} = \frac{C}{C_0 - C}$$

where C_0 and C represent the initial and equilibrium concentrations of IFM in the octanol phase.

Incubation of IFM in GI tract contents and 80% plasma

Hydrolysis of IFM in contents of different segments of the GI tract

Rats weighing 200–220 g were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). All experimental procedures carried out in this study were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Shenyang Pharmaceutical University. Nonfasting rats were anesthetized with diethyl ether and a midline incision was made. The stomach and intestine segments were removed. The luminal contents of the stomach, small intestine, cecum, and colon were removed and transferred to cold isotonic buffer solutions (pH 4.5, 6.8, 7.4, and 7.4, respectively, to simulate the environments of the rat's GI tract), which were then homogenized for 5 minutes to give a final homogenate concentration of 0.1 g/mL. After passing through a gauze filter to remove large particulate material, a 10 mL portion of homogenate was transferred to a test glass, to which IFM (0.2 mg) was added and then incubated on a water bath at 37°C. At predetermined time points, 1.0 mL samples were withdrawn and heated on a water bath at 100°C for 1 minute to stop the enzymatic hydrolysis. To the supernatant obtained by centrifugation, 50 μL dilute hydrochloric acid solution (0.01 M), 50 μL internal standard solution (10 μg/mL), and 2.5 mL isopropanol were added. The mixture was then vortexed and centrifuged. The supernatant was evaporated to dryness under nitrogen gas. The residue was dissolved in mobile phase, centrifuged, and 20 μL of the supernatant was injected into the HPLC. Each experiment was carried out in triplicate.

Hydrolysis of IFM in 80% plasma

An aliquot of IFM solution (35 μM) in acetonitrile and 6 mL of 80% rat plasma (pH 7.4, prepared by mixing 80 volumes of plasma and 20 volumes of phosphate buffer) were mixed and incubated at 37°C with stirring. At appropriate intervals, 0.2 mL samples were removed and heated on a water bath at 100°C to stop further hydrolysis. To the supernatant obtained by centrifugation, 50 μL dilute hydrochloric acid solution (0.01 M), 50 μL internal standard solution (10 μg/mL), and 2.5 mL isopropanol were added. The mixture was then vortexed and centrifuged and the supernatant was evaporated to dryness under nitrogen gas. The residue was dissolved in mobile phase, and 20 μL of the supernatant was injected into the HPLC.

For the determination of 5FU, isopropanol was replaced by ethyl acetate as the extracting agent for biological samples. 5-Bromouracil and diclofenac sodium were used as the internal standards for 5FU and IFM, respectively. Quantitation was achieved by the peak area ratios of the drug to the internal standard.

Preparation of IFM dry emulsion

IFM (2.5 g) and Tween-80 (3.75 g) were mixed with MCT (6.25 g) to form a uniform oil mixture. With grinding, water (2.5 g) was added and then homogenized to obtain a primary emulsion.

Then, 10.0 g gum acacia and 10.5 g CMC-Na were added bit by bit to the obtained primary emulsion with stirring. The mixture was transformed from a fluid to a paste and finally to a free-flowing powder, which was the IFM-dry emulsion.

Reconstitution and characterization of the dry emulsion

Reconstitution of liquid emulsions was performed by suspending 1.0 g of powder in 10.0 mL of distilled water in a vial. Then, the emulsion was shaken manually for 5 minutes, and an aliquot was withdrawn for further characterization.

The surface morphology of the liquid emulsion was observed under a microscope (XSP-16A model). The droplet size distribution of the emulsions after reconstitution was determined by light diffraction using a Beckman Coulter LS230 laser sizer. The droplet size distribution was determined on the basis of the average volume distribution.

In situ intestinal perfusion studies

Preparation of perfusion solution

The perfusion solution was a Krebs–Ringer buffer solution containing 133 mM NaCl, 4.7 mM KCl, 2.3 mM $MgCl_2$, 3.3 mM $CaCl_2$, 1.8 mM NaH_2PO_4, 16.4 mM $NaHCO_3$, and 7.8 mM D-glucose in purified water.

An appropriate amount of IFM dry emulsion was dispersed in Krebs–Ringer buffer solution to produce IFM with different concentrations as 2, 5, and 10 μg/mL.

The stability of IFM in Krebs–Ringer buffer solution

The study was carried out by adding 1 mL of IFM solutions (1 mg/mL acetonitrile) to 10 mL of Krebs–Ringer buffer solution. The solutions were incubated at 37°C and shaken at 50 rpm and sampled (0.5 mL) at specified times up to 2 hours. The samples were diluted properly with mobile phase and then analyzed by HPLC to determine the remaining IFM. Each experiment was performed in triplicate.

Pseudo-first-order hydrolysis rate constants (k) were determined from linear plots of $\ln(C_t - C_\infty)$ versus time, where C_t and C_∞ are the concentrations of IFM at time t and ∞, respectively. The slopes ($-k$) of linear plots were determined by linear regression.

In situ intestinal perfusion experiment

The experiment was carried as described by Stewart et al[32]. Rats were fasted for approximately 18 hours with free access to water before surgery. After anesthesia through intraperitoneal administration of thiopental sodium (50 mg/kg), rats were placed on a heating pad to maintain their body temperature at 37°C. The intestine of the rats was exposed by a midline abdominal incision and the whole small intestine from the beginning of duodenum to the end of ileum was cannulated with glass tubing. The selected segment was rinsed with phosphate-buffered saline (10 mL) to clear the gut and the perfusion solution maintained at 37°C was infused at a flow rate of 5 mL/min into the segment for 10 minutes to achieve a steady state. The volume in circulation was recorded as the 0-minute volume, and the flow rate was adjusted to 2.5 mL/min. Serial samples (0.5 mL) were collected from the segments at predetermined time intervals (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, and 2.0 hours), the volume in circulation was recorded, and then replaced with 0.5 mL Krebs–Ringer solution. Samples were filtered and then analyzed by HPLC. Here, the method of direct volume recording was used[21].

The absorption rate constant (K) was calculated from the following equation:

$$\ln X = \ln X_0 - K_t$$

where X is the amount of IFM in the perfusate at t and t is the predetermined time point.

Results and discussion

Synthesis and characterization of indomethacin-5-fluorouracil-methyl ester

Figure 1 represents the reaction between IDM and 5FU. The prodrug approach, in which a derivative of the active compound is synthesized, has been proven to be an effective and important way of overcoming various absorption, distribution, metabolism, and excretion barriers that restrict the application of many chemical agents as orally administered drugs[22].

Although 5FU is one of the most commonly used drugs to treat many types of cancer, oral administration of FU results in variable bioavailability. Many prodrugs of 5FU have been synthesized to alter the physicochemical property and hence the absorption of 5FU.

IDM is a typical NSAID widely used for indications ranging from inflammation and pain to cardiovascular and genitourinary diseases. However, GI side effects often occur[14]. The IDM molecule possesses an active carboxyl group and is an ionic surface active compound in the soluble form. Such compounds have long been known to adversely injure the mucosa of the GI tract[33]. However, a series of IDM derivates can be synthesized involving combination of the carboxyl group with other agents to minimize these side effects[34].

In this study, to take advantages of the synergia effect of two drugs for cancer therapy and minimize the GI tract disturbance induced by alone administration, we synthesized a novel combination of 5FU and IDM, which is an ester. First, 1,3-dimethylol-5-fluorouracil was synthesized from formaldehyde and 5FU to form a hydroxyl-containing intermediate product.

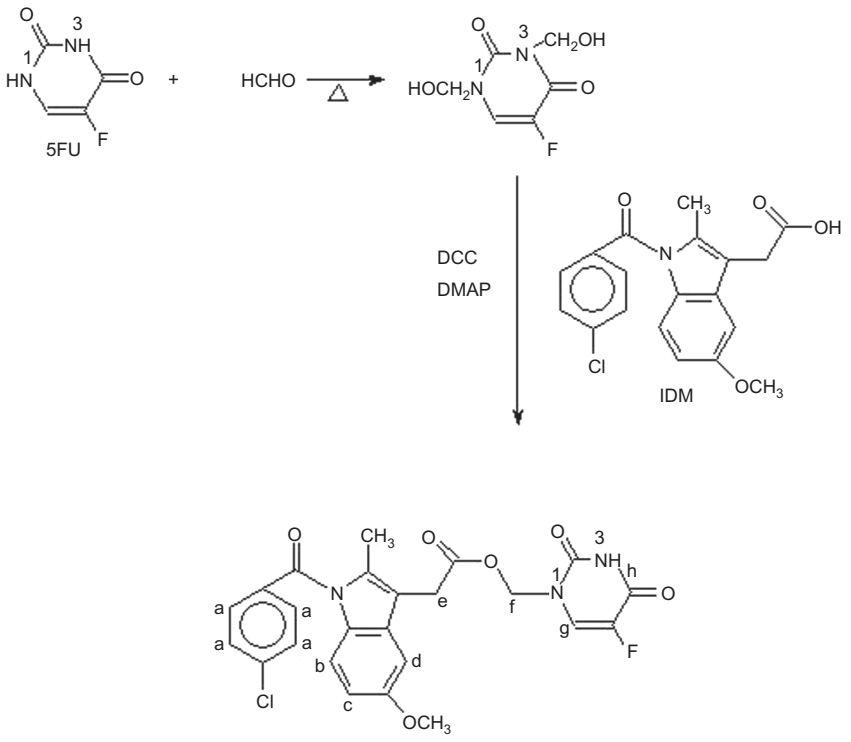

Figure 1. Schematic representation of the reaction mechanism.

In the presence of *N*,*N*-Dicyclohexylcarbodiimide and DMAP, the carboxyl group of IDM reacted with the hydroxyl group of the intermediate product to form a new ester[23].

In theory, three compounds should be obtained because the N_1 and N_3 positions are all active in the 5FU molecule. In practice, three spots were observed when thin-layer chromatography was performed to monitor the reaction progress on silica-gel plates (Merck silica-gel G). However, the N_1 position is more active and has less steric hindrances, compared to the N_3 position in 5FU molecule. So, under the experimental conditions used, we mainly obtained IFM, which is the conjugate of 5FU at the N_1 position and IDM in the same molar ratio. The other two conjugates were negligible and lost during purification.

The melting point was determined as 217°C. The purified IFM had UV absorbance maxima at 259 and 317 nm in acetonitrile.

IR (KBr) cm^{-1}:3199 (Ar–H), 3437 (N–H), and 1732 (O–C=O) (Figure 2).

^{1}H-NMR (DMSO-d_6) δ:3.84 (s, 2H, e position), 5.62 (s, 2H, f), 6.72 (d d, 1H, c), 6.92 (s, 1H, g), 7.01 (d, 1H, d position), 7.66 (s, 4H, a position), 8.11 (d, 1H, b position), and 12.02 (s, 1H, h position) ppm (Figure 3).

MS (m/z): $[M-1]^+ = 499$ (Figure 4).

Based on the above characterization results, we concluded the formation of the new compound IFM.

Water solubility and apparent partition coefficient studies

The results of the solubility studies of IFM are presented in Table 1. The data show that IFM is more soluble in organic solvents, especially acetonitrile and tetrahydrofuran (THF), compared with water. IFM was found not to be soluble in water and buffer solutions with the assay method that we used.

High lipophilicity is often required because it can affect the absorption, distribution, metabolism, and excretion properties of drugs in biological systems. To obtain a better understanding of the overall properties of the compound, its lipophilicity, expressed as the 1-octanol/water partition coefficient, was determined under two different pH conditions. The apparent partition coefficients represent the distribution of both ionized and nonionized drug molecules between the two phases. As is to be expected, the apparent partition coefficient varies with the pH of the aqueous solution. For ionizing compound, it is pH dependent. The apparent partition coefficient of IFM in 1-octanol/phosphate-buffered saline (pH 1.2 and 7.4) was found to be 260.1 and

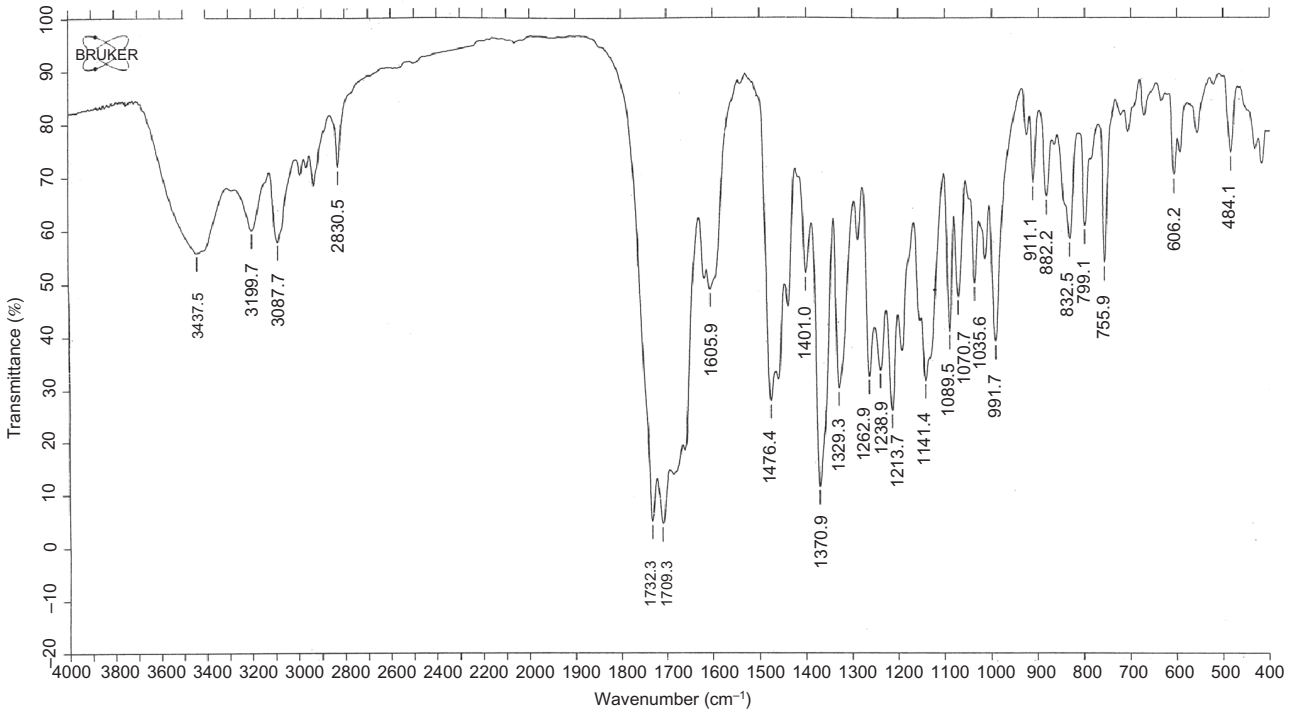

Figure 2. IR spectrum of IFM.

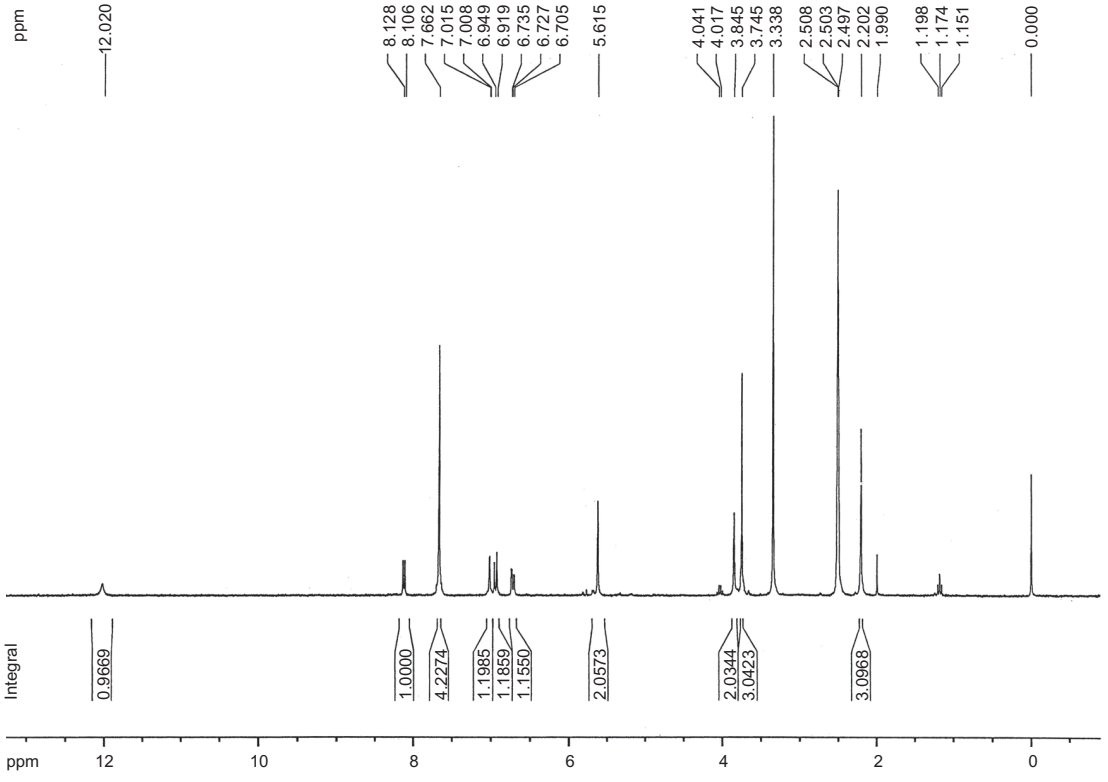

Figure 3. ^{1}H-NMR spectrum of IFM.

350.7, respectively. The partition coefficient study of IFM showed that the major fraction of IFM partitioned into the organic phase, which indicates an increased lipophilicity compared to that of parent compound 5FU as reported previously[24]. Furthermore, the different log P_{app} values demonstrated the pH dependence of IFM.

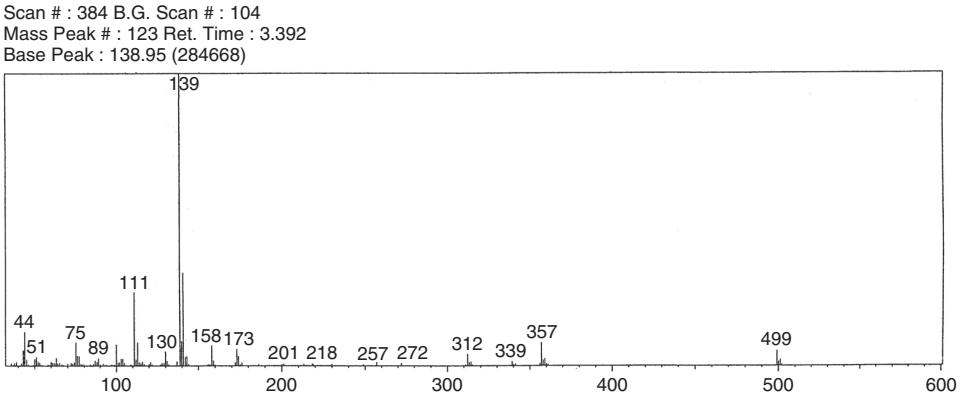

Figure 4. MS spectrum of IFM.

Table 1. Solubility of IFM in different solvents.

Solvent	Solubility (μg/mL)	Solvent	Solubility (μg/mL)
Ethanol	62.36	Chloroform	52.47
Isopropanol	26.62	THF	2167
Acetonitrile	2854	1-Octyl alcohol	26.19
Ether	46.40	Benzene	66.93

Incubation of IFM in GI tract contents and 80% plasma

The incubation of IFM in the contents of different segments of the GI tract and in plasma was carried out to evaluate the hydrolysis of IFM under physiological conditions.

Figure 5 shows the concentration–time profile for the remaining IFM and released 5FU during incubation of IFM in contents of stomach, colon, cecum, and small intestine over 4 hours. Less than half of IFM was hydrolyzed in 4 hours in the presence of the contents of stomach, cecum, and colon segments. However, more than half of IFM was hydrolyzed in 4 hours in the presence of the contents of the small intestine, which may be attributed to the existence of ester enzyme in small intestine[25]. 5FU and IDM could be released slowly in each segment of the GI tract, as also can be seen from the figure, which may be helpful in minimizing the side effects of IDM on the GI tract. However, to further protect IFM from degradation in GI tract and enhance the absorption of IFM intact, a suitable delivery system for IFM may be considered.

Hydrolysis studies of IFM in 80% human plasma were carried out to evaluate the release of 5FU after absorption into the systemic circulation. As can be seen from Figure 6, more than half of IFM was converted to 5FU within 5 minutes. This indicates that IFM is very instable in plasma and releases FU rapidly, which may also be promoted by the enzyme effect in plasma.

Preparation, characterization, and reconstitution of IFM dry emulsion

According to the studies described above, we concluded that IFM was more liposoluble than water soluble. We found that IFM has a low aqueous solubility and, hence, a poor oral bioavailability when administered as a conventional tablet or capsule.

Incorporation of a poorly water-soluble drug in an oil-in-water emulsion, wherein the drug is dissolved or dispersed, has been reported to improve the bioavailability of a range of insoluble drugs[26]. The increased bioavailability is caused by elimination of the dissolution step or an increased dissolution rate of the dispersed drug. In addition, the lipid digestion products may contribute to solubilization of the drug molecules during transport to the unstirred water layer of the intestinal membrane[27,28].

Dry absorbed emulsion is a complex system initiated by a water-in-oil (w/o) emulsion, which is changed into a free-flowing powder by using adsorbents with suitable polarities[29]. We prepared IFM dry emulsion with an MCT as the lipid material and gum acacia and CMC-Na as the adsorbent and solid carrier, respectively, to enhance the solubility and hence the absorption of intact IFM after oral administration.

The surface morphology and droplet size distribution of the liquid emulsion after reconstitution were examined by microscopy and the Beckman Coulter LS230 laser sizer. Figure 7 shows the surface morphology of the reconstituted lipid emulsions. The dry emulsion was efficiently reconstituted in water, and the mean droplet size was 2.416 μm (SD = 0.047).

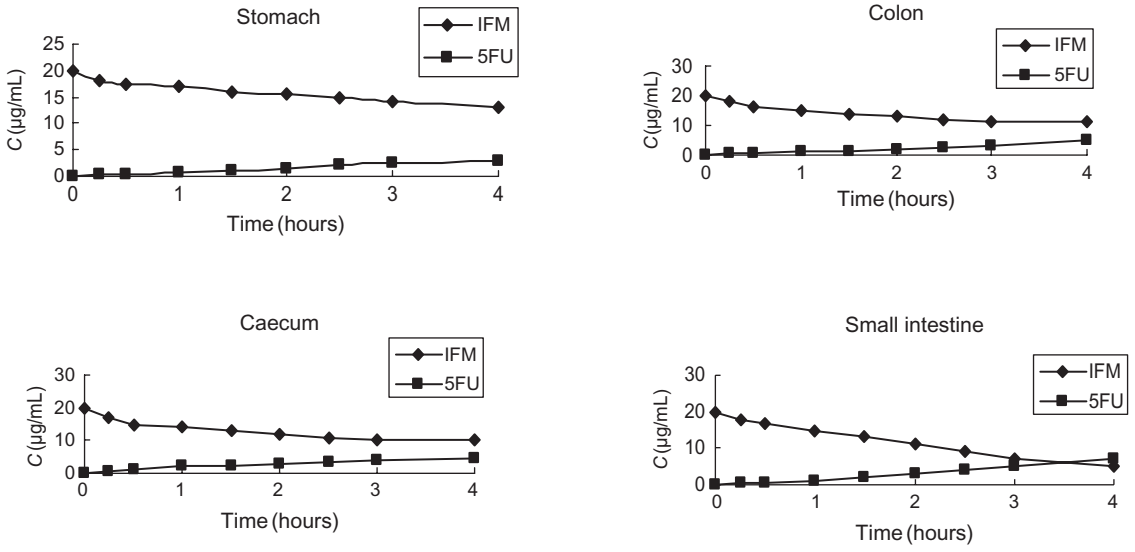

Figure 5. Concentration–time profile for IFM and 5FU during incubation of IFM in contents of different segments of the GI tract over 4 hours.

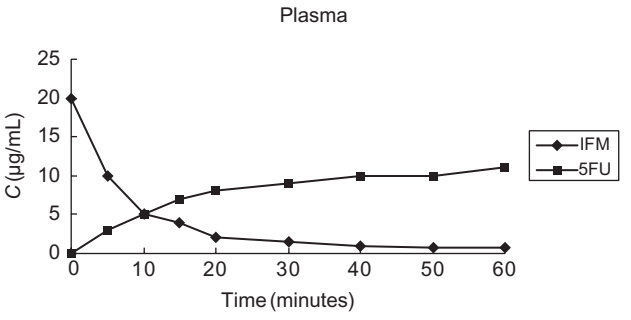

Figure 6. Concentration–time profile for IFM and 5FU during incubation of IFM in rat plasma (80%) over 1 hour.

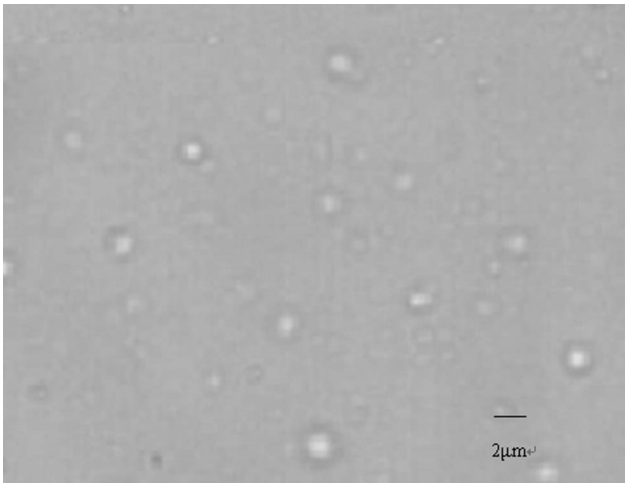

Figure 7. Microscopy of IFM emulsion after reconstitution in water (×1500).

In situ intestinal perfusion studies

The incubation of IFM in Krebs–Ringer solution was carried out to evaluate the stability of IFM in perfusion solution as a basis for the in situ intestinal absorption studies.

Generally, the hydrolysis of ester drugs follows the pseudo-first-order kinetic equation. The hydrolysis kinetic equation for IFM in Krebs–Ringer solution was as follows: $\ln(C_\infty - C_t) = -0.054t + 0.9703$. The hydrolysis rate constant (k) was calculated as 0.054/h.

The intestinal absorption kinetic equations of IFM dry emulsion (2, 5, and 10 μg/mL) were as follows: $\ln X = \ln X_0 - 0.473t$, $\ln X = \ln X_0 - 0.423t$, and $\ln X = \ln X_0 - 0.433t$, and the absorption rate constant K was 0.473, 0.423, and 0.433/h, respectively.

Comparison of the absorption rate constant, *K,* with the hydrolysis rate constant, *k,* showed that IFM was absorbed effectively in the GI tract in situ intestinal perfusion experiment. The absorption, not the degradation, of IFM accounted for the reduction of IFM during the intestinal perfusion process. This may be attributed to the solubilization and enhanced adsorption of insoluble drugs by lipid emulsions[30].

To understand the absorption mechanism of IFM dry emulsion through the GI tract, three different concentrations of IFM dry emulsions (2, 5, and 10 μg/mL) were administered. The in situ intestinal absorption curve of IFM dry emulsion with different concentrations is shown in Figure 8.

Comparison of the absorption kinetics of IFM dry emulsion with different concentrations showed a concentration-independent absorption for IFM. The results indicated that the intestinal absorption of IFM

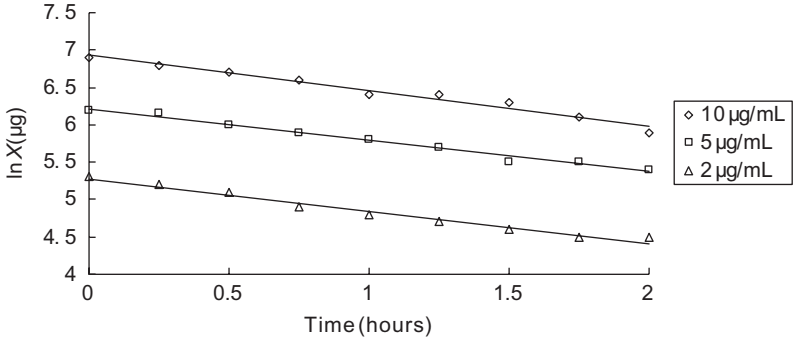

Figure 8. The in situ intestinal absorption curve for IFM dry emulsion with different concentrations over 2 hours.

was by passive diffusion across the biological membranes, the same with 5FU as reported previously[31].

Nonabsorbed makers, such as a dye (e.g., phenol red) or a radioactive isotope (e.g., ^{14}C-PEG-3500), are usually added to the perfusion solutions to determine the volume change because of water absorption in intestinal perfusion experiments. However, phenol red has been reported to be partially absorbed in the small intestine and this might interfere with the transport of some drugs, especially poorly soluble ones. Use of radiolabeled isotopes raises safety concerns. So, to overcome these disadvantages, many simpler methods have been adopted, such as gravimetric analysis and direct recording of the volume.

Conclusion

In this study, we developed an IFM dry emulsion for oral delivery of 5FU. Physicochemical properties of the newly synthesized compound demonstrated the formation of IFM. Incubation of IFM in the contents of the GI tract and rat plasma showed that IFM was not relatively stable in the GI tract contents during the transit of the drug through the GI tract, whereas it was very unstable in plasma and 5FU was released rapidly. IFM dry emulsion was easily reconstituted in water, and the in situ intestinal perfusion studies showed that IFM could be absorbed effectively from the dry emulsion formulation. The absorption mechanism of IFM dry emulsion through the GI tract was by passive diffusion. In conclusion, the IFM dry emulsion represents a promising oral delivery system for 5FU, in that it may help to produce combined effects of IDM and 5FU for cancer therapy, reduce the side effects of IDM on the GI tract, and improve the oral adsorption of 5FU. Further studies are planned to examine the in vivo behavior of the IFM dry emulsion and solve the problem of catabolic and anabolic metabolism of 5FU in systemic circulation.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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