Characterization and evaluation of an oral microemulsion containing the antitumor diterpenoid compound ent-11α-hydroxy-15-oxo-kaur-16-en-19-oic-acid

Abstract: The objective of this study was to develop an oral microemulsion formulation of the antitumor diterpenoid agent, ent-11α-hydroxy-15-oxo-kaur-16-en-19-oic-acid (henceforth referred to as 5F), to enhance its bioavailability and evaluate its hepatotoxicity. Pseudoternary phase diagrams showed that the optimal microemulsion formulation contained 45% water, 10% castor oil as the oil phase, 15% Cremophor EL as the surfactant, and 30% as a cosurfactant mixture of 1,2-propanediol and polyethylene glycol (PEG)-400 (2:1, w/w). The microemulsion preparation was characterized and its droplet diameter was within 50 nm. Release of 5F in vitro from the microemulsion was slightly increased compared with a suspension containing the same amount of active drug. Pharmacokinetic parameters in vivo indicated that bioavailability was markedly improved, with the relative bioavailability being 616.15% higher for the microemulsion than for the suspension. Toxicity tests showed that the microemulsion had no hepatotoxicity in mice. These results suggest the potential for 5F microemulsion to be administered by the oral route.

Keywords: antitumor, diterpenoid, microemulsion, pharmacokinetics, toxicity

Introduction

Pteris semipinnata L (Banbianqi in Chinese) is a traditional medicinal herb widely used as a folk medicine in the treatment of diarrhea and a variety of inflammatory conditions. An ent-kaurane diterpenoid compound, ent-11α-hydroxy-15-oxo-kaur-16-en-19-oic-acid, is isolated from this herb. For simplicity, this compound is known as 5F, and its chemical structure is shown in Figure 1. It has been found that 5F is able to inhibit growth of several tumor cell lines, including gastric adenocarcinoma (MGC-803) cells, lung adenocarcinoma (SPC-A-1) cells, human promyelocytic leukemia (HL-60) cells, nasopharyngeal carcinoma (CNE-2Z) cells, and liver adenocarcinoma (BEL-7402, HepG2) cells, and that 5F induces apoptosis in human cancer cells in a concentration-dependent and time-dependent manner. The mechanism underlying the pharmacological effects of 5F has been studied in cell culture, and the compound is reported to inhibit NF-kB significantly, leading to a decrease in Bcl-2 and an increase in Bax and Bak.

Experiments in vitro and in vivo show that 5F is worth developing commercially as a novel antineoplastic drug. However, this type of ent-kaurane compound is usually cytotoxic, in particular causing hepatic lesions because it is metabolized mainly in the liver. Moreover, its poor aqueous solubility contributes to high variability in absorption. These shortcomings limit the application of 5F as a therapeutic agent.
In recent years, efforts have been made to reduce its toxicity and improve its bioavailability, eg, by developing a sodium salt solution of 5F for injection,\(^\text{11}\) which unfortunately was found to be irritative and painful in laboratory animals. The oral route is the most physiologically beneficial and is easily accepted by patients. Therefore, it is necessary to develop an oral preparation of 5F that can overcome the above problems and have a beneficial therapeutic effect.

Microemulsions have recently attracted much attention in pharmaceutical research.\(^\text{12,13}\) Their high thermodynamic and kinetic stability, low viscosity, and optical transparency make them very attractive for pharmaceutical application in improving the solubility and oral absorption of drugs that are poorly water-soluble.\(^\text{14}\) Microemulsions are spontaneously composed of surfactant, cosurfactant, oil, and water, with a particle size of less than 100 nm in diameter. Microemulsions including surfactants and oils have the advantages of low free energy and a large surface area, which are necessary for transporting drugs to the gastrointestinal tract membrane for absorption.\(^\text{15,16}\)

The purpose of the present work was to develop an oral microemulsion containing 5F. The optimal microemulsion formulation was composed of castor oil, Cremophor EL, a mixture of 1,2-propanediol/polyethylene glycol (PEG)-400, and distilled water. The physicochemical characteristics and in vivo toxicity and pharmacokinetic characteristics of this microemulsion were also investigated.

## Materials and methods

### Materials

The compound 5F (purity > 96%, by high-performance liquid chromatography) was isolated and purified by our laboratory and identified by Professor Pan Wenlong (Institute of Chemistry Chinese Academy of Science, Guangzhou, People’s Republic of China). Cremophor EL was purchased from Sigma (St Louis, MO, USA). 1,2-propanediol and PEG-400 were supplied by Haiwang Fine Chemical Co, Ltd (Tianjin, People’s Republic of China). All other reagents and solvents used were of analytical grade and water was of Milli-Q grade.

Chinese Kunming mice (body weight 23–27 g) were purchased from Guangdong Medical College Laboratory Animal Center (Zhanjiang, People’s Republic of China) and maintained in a light-controlled room at a temperature of 22°C ± 2°C and relative humidity of 55% ± 5%. All the animals were pathogen-free and allowed free access to food and water. All care and handling of the animals was performed with the approval of the Institutional Authority for Laboratory Animal Care of Guangdong Medicinal College.

### Solubility studies

Determining an optimal microemulsion composition was necessary to achieve maximum solubility, so the solubility of 5F in various oils, surfactants, and cosurfactants was determined. An excess amount of 5F was added to 1 mL of each selected vehicle in a centrifugal tube, followed by mixing (100 rpm) in a shaking incubator (Jinke Technology, Shanghai, People’s Republic of China) at 25°C for 24 hours. The samples were centrifuged at 13,000 rpm for 10 minutes to remove the excess 5F, after which the concentration of 5F in the supernatant was measured by high-performance liquid chromatography after appropriate dilution with methanol.\(^\text{11}\)

### Preparation of pseudoternary phase diagram and microemulsion

Pseudoternary phase diagrams were utilized to determine regions of microemulsion formation, from which a large number of potential microemulsions were identified, and the formulation of an oil/water (o/w) microemulsion was screened for optimal solubilization of the lipophilic components. Based on the results of the solubility study (Table 1), castor

### Table 1 Solubility of 5F in various solvents at 25°C (n = 3)

| Solvents                        | Solubility (μg/mL) |
|---------------------------------|--------------------|
| Oil                             |                    |
| Soybean oil                     | 171.94 ± 12.32     |
| Castor oil                      | 1907.14 ± 36.44    |
| Medium-chain triglycerides      | 724.42 ± 24.64     |
| Oleic acid                      | 1846.24 ± 28.26    |
| Surfactant                      |                    |
| Tween-80                        | 3542.22 ± 66.36    |
| Isopropyl myristate             | 1028.36 ± 52.34    |
| Cremophor EL                    | 21511.75 ± 122.26  |
| Labrasol                        | 28762.66 ± 156.34  |
| Cosurfactant                    |                    |
| 1,2-propanediol                 | 3200.76 ± 74.42    |
| Alcohol                         | 26368.82 ± 64.42   |
| PEG-400                         | 8697.18 ± 15.34    |
| Plurol oleique                  | 3024.68 ± 56.38    |

Abbreviation: 5F, ent-11α-hydroxy-15-oxo-kaur-16-en-19-oic-acid.
oil was selected as the oil phase, and the nonionic surfactant, Cremophor EL, was chosen as the surfactant to form the microemulsion. PEG-400 was chosen as the cosurfactant to form microemulsions because of its safety and biocompatible characteristics. Pseudoternary phase diagrams of oil, surfactant, cosurfactant, and water were developed in order to formulate a series of microemulsion delivery systems for 5F. Briefly, 5F was dissolved in 1,2-propanediol, the surfactant was mixed with the cosurfactant (1,2-propanediol and PEG-400 at a mass ratio of 2:1 and 1:1, respectively) in fixed mass ratios (Km, mass ratio of surfactant to cosurfactant) of 1:2 and 1:3. The weight ratios of the oil to surfactant-cosurfactant mixture were varied from 1:9 to 9:1 (w/w). Under moderated magnetic stirring, the mixtures of oil, surfactant, and cosurfactant at certain weight ratios were diluted with water dropwise. After being equilibrated, the mixtures were assessed as microemulsions when they were clear in appearance. Under moderated magnetic stirring, the mixtures of oil, surfactant, and cosurfactant at certain weight ratios were diluted with water dropwise. After being equilibrated, the mixtures were assessed as microemulsions (when the appearances of the mixtures were changed from turbid to clear and colorless). The data of adding water were recorded and marked in the pseudoternary phase diagram. Based on the critical values obtained at different mass ratios, the phase boundary was determined.

**Physicochemical characterization of o/w microemulsion**

The morphology of the microemulsions was examined using an energy-filtering transmission electron microscope (JEM-2010HR, JEOL, Tokyo, Japan) with an accelerating voltage of 80 kV. The microemulsions were negatively stained with 2% sodium phosphotungstate (pH 7) and placed on carbon-coated 400 mesh copper grids, followed by drying at room temperature before measurement.

A given volume of microemulsion was diluted with saline to a definite volume in a flask and shaken gently to mix thoroughly. Samples were then passed through a 0.22 µm pore size filter to remove dust particles before size measurement. The particle size of the microemulsion formed was determined by dynamic light scattering (Zetasizer ZEN 3600, Malvern, Worcestershire, UK) with a scattering angle of 90 degrees at 25°C.

**In vitro release study**

In vitro release of the 5F microemulsion was tested using a dialysis method reported in the Chinese Pharmacopoeia (2010 version, V XD74). 10 mL each of the 5F microemulsion and 5F suspension (control) containing a given amount of drug was placed into a dialysis bag (molecular weight cutoff 8000–10,000, Spectrum Medical Industries Inc, Laguna Hills, CA, USA). The dialysis bag was then firmly sealed and immersed in a flask containing 300 mL of release medium (pH 6.5 phosphate-buffered solution) which was kept in a shaking water bath at 37°C and 100 rpm. At predetermined intervals, 1 mL aliquots of the release medium were withdrawn and immediately replaced with an equal volume of fresh medium. Based on the amount of drug released, as determined by high-performance liquid chromatography, the percent cumulative release of 5F was calculated and compared with that released from the 5F suspension containing the same quantity of drug.

**In vivo pharmacokinetic studies in mice**

Ninety mice were randomly assigned to two groups for intragastric administration of 5F suspension or 5F microemulsion at a dose of 20 mg/kg body weight. Groups of five mice per formulation per time point were used in this study. At time points of 0, 0.16, 0.33, 0.5, 1, 2, 3, 4 and 8 hours, 1.5 mL blood samples were collected from the ocular artery of the five mice in each group, after eyeball removal, and placed in heparinized test tubes. The plasma was immediately separated by centrifugation at 3000 g for 10 minutes and stored at −80°C until use.

The liquid-liquid extraction procedure for the plasma samples was executed as follows: 200 µL of plasma was added to 20 µL of 2 mol/L acetic acid and 2 mL ethyl acetate. The mixture was vortexed for one minute and centrifuged at 3000 g for 10 minutes. The organic phase was then transferred into another tube and evaporated to dryness at 35°C with the aid of a gentle stream of air. The residue was dissolved in 100 µL of mobile phase, and a 20 µL aliquot was injected into a high-performance liquid chromatography system for analysis.

A modified high-performance liquid chromatography/ultraviolet method was used to determine 5F levels in the mouse plasma. Liquid chromatography was carried out on an Agilent 1200 series high-performance liquid chromatography apparatus which consisted of a quart pump, a degasser, a Rheodyne 7125 injector with a 20 µL loop (Rheodyne Inc, Cotata, CA, USA), an ultraviolet detector, a column oven, and an LC1200 workstation (Agilent, Santa Clara, CA, USA). The separations were performed on a Hypersil ODS C18 250 × 4.6 mm column with a 5 µm particle size (Thermo Scientific, Waltham, MA, USA). The mobile phase consisted of a mixture of 0.5% (v/v) acetic acid solution and methanol (45:55, v/v) at a flow rate...
of 1.0 mL per minute. The mobile phase was prepared daily and filtered under vacuum through a 0.45 µm membrane filter before use. The eluate was continuously monitored at a wavelength of 242 nm. Separations were maintained at 35°C.

Pharmacokinetic parameters, including area under the drug concentration-time curve (AUC), time taken to reach peak plasma concentration (T<sub>max</sub>), peak plasma concentration reached (C<sub>max</sub>), mean residence time (MRT), and apparent clearance (CL/F) were calculated using Drug and Statistics software version 3.0 (TCM, Shanghai, People’s Republic of China).

**In vivo toxicity studies**

**Biochemical markers of liver injury**

Hepatotoxicity was investigated by quantitative analysis of glutamic oxaloacetic transaminase activity, which is often used as a biochemical marker of liver injury. Glutamic oxaloacetic transaminase activity was measured by conversion of aspartic acid and ketoglutaric acid to glutamic acid and oxaloacetic acid using an autoanalyzer (Shimadzu CL-7200, Tokyo, Japan).

**Histological staining of liver tissue**

The experiment groups of mice were orally administrated with 5F suspension and 5F microemulsion, the control group was treated with saline, and the positive model group was treated with triptolide at the dosage of 0.4 mg per kg body weight. The liver tissue was subsequently removed from the treated mice, a small portion (about 200 mm<sup>3</sup>) of which was fixed in 10% buffered formalin and stained with hematoxylin and eosin for histological examination using conventional methods.

**Statistical analysis**

All the data are presented as the mean ± standard deviation. The statistical significance of differences was determined by the Statistical Package for Social Sciences for Windows version 13.0 (SPSS Inc, Chicago, IL, USA).

**Results and discussion**

**Solubility studies**

Solvents used in a microemulsion should be nontoxic and enable a high degree of drug solubility. Furthermore, for application in the pharmaceutical industry, they should also be inexpensive and easily available, so oils, surfactants, and cosurfactants were investigated as materials for forming a microemulsion in this study. The solubility of 5F in various solvents is shown in Table 1. Solubility in castor oil was the highest among all the oils tested, and that in Cremophor EL was much higher than in the other oil phases (Labrasol was much higher than in the other solvents, but Labrasol is more costly, therefore Cremophor EL was selected as surfactant for both advantages of good solubility and low cost). 1,2-propanediol and PEG-400 were selected as cosurfactants because the solubility of 5F in both was high. Ethanol was not used as a cosurfactant because of its volatile nature. The optimal 5F microemulsion consisted of castor oil, Cremophor EL, 1,2-propanediol, and PEG-400, in which the solubility of 5F was higher than in the others, and therefore would have enhanced bioavailability.

**Pseudoternary phase diagram studies**

The water titration method was used for construction of a pseudoternary phase diagram in order to identify an existing range of microemulsions and form an o/w microemulsion with optimal solubilization of the lipophilic component. It was found that the surfactant/cosurfactant blend of Cremophor EL:1,2-propanediol/PEG-400 (2/1, 1/1) in ratios of 1:2 and 1:3 was able to incorporate the largest amount of water when combined with castor oil. The pseudoternary phase diagram obtained is shown as Figure 2.

The areas to the right of the boundary lines were transparent o/w microemulsion regions and to the left were turbid emulsion regions, which are not monophasic. The
area of the microemulsion region decreased with decreasing ratios of 1,2-propanediol to PEG-400 when Km was constant, indicating that a decrease in the short-chain alcohol content is adverse to the incorporation of oil in a microemulsion. In addition, the area of the microemulsion region became larger with increasing Km at each ratio of 1,2-propanediol to PEG-400. A cosurfactant is beneficial when forming a microemulsion at an appropriate concentration range. However, an excessive amount of cosurfactant would cause the system to become less stable because of its intrinsic high aqueous solubility and lead to an increase in droplet size.

Hence, the ratio of surfactant to cosurfactant selected was 1:2 rather than 1:3.

Appropriate solvents should have good solubilizing capacity for the drug substance, which is essential for constructing a microemulsion. Castor oil was used as the oil phase because the solubility of 5F in castor oil was much higher than in the other oils (Table 1).

Nonionic surfactants are used in most investigations of microemulsion drug delivery systems because they are less toxic and less affected by pH and ionic strength. Cremophor EL is a good nonionic emulsifier, and can reduce surface tension and fluidize the interfacial surfactant film, which can expand the area of the microemulsion system. Moreover, Cremophor EL is a medium-length alkyl chain surfactant with a high hydrophilic-lipophilic balance value (14), which may contribute to the formation of an o/w microemulsion because it is generally accepted that surfactants with a high hydrophilic-lipophilic balance value (8–18) are preferred for the formation of o/w microemulsion systems.

With regard to the selection of a cosurfactant, 1,2-propanediol and PEG-400 have relatively high hydrophilicity, and both provided high drug solubility of all the vehicles tested in the solubility studies. Therefore, it was reasonable to select these as the cosurfactant. When certain proportions of the oil phase and surfactant were fixed, the microemulsion area using PEG 400 was the largest and the solubility of 5F was improved to 20.22 mg/mL; when the solubility of 5F in water was 0.024 mg/mL, the solubility of 5F in PEG-400 improved about 100-fold. The optimal ratio solubility of 5F in PEG-400 improved about 100-fold. The optimal ratio of surfactant to cosurfactant selected was 1:2 rather than 1:3.

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Based on these results, the optimal composition required to form the microemulsion was 45% water, 10% castor oil as oil phase, 15% Cremophor EL as the surfactant, and 30% as a cosurfactant mixture of 1,2-propanediol and PEG-400 (2:1).

Physical characterization of microemulsion
The microemulsion droplets obtained were spherical and relatively even in size, as shown in Figures 3 and 4. The average droplet size of the 5F microemulsion was within 50 nm. Droplet size has been thought to have an effect on drug absorption, ie, the smaller the droplet size, the larger the interfacial surface area. The effect of the medium on droplet size was also investigated in our study. When the microemulsion was respectively diluted 100-fold in distilled water, 0.9% NaCl, 0.1 mol/L HCl, or pH 6.8 phosphate buffer, there was no significant difference in the resulting droplet size or spherical shape among the four different types of medium, indicating that the microemulsion would not be affected by pH in the gastrointestinal tract.

In vitro release
The ability of the microemulsion to deliver 5F was investigated by determining the drug release rate in vitro. The drug release profile is shown in Figure 5. Obviously, the release of drug from the suspension was slightly faster during the first hour than that from the microemulsion. In the suspension, a small amount of drug in free molecular form can pass rapidly through the membrane by molecular diffusion. In contrast, the drug molecules were covered by droplets in the microemulsion, which were found to be a physical barrier impeding movement of the drug molecules into the receiving solution.

In spite of the slightly greater release of drug from the suspension than from the microemulsion in the first hour, the release of drug from the microemulsion was mildly increased compared with that from the drug suspension in the following
6 hours. Moreover, no burst release occurred for any of the samples during the initial period. This finding might be attributed to several factors, including droplet size and the length of the PEG chain in the surfactant. The small droplet size contained in the microemulsion enabled better adherence to the membrane during transport of the drug and optimized its permeation, and the surfactant, Cremophor EL, decreased the surface tension of the two immiscible liquids (water and oil), which was advantageous for releasing the drug from the droplets. Moreover, PEG-400 was short in chain length, so could increase drug permeation across the membrane. Therefore, the release of 5F was slightly different between the two delivery systems.

**In vivo pharmacokinetic studies**

The plasma concentration-time profiles for the 5F suspension and 5F microemulsion in Kunming mice are shown in Figure 6, and the main pharmacokinetic parameters calculated based on a noncompartment analysis method are summarized in Table 2.

There was no significant difference in $C_{\text{max}}$ between the microemulsion (3.12 µg/L) and the suspension (3.17 µg/L). The MRT of the microemulsion (2.451 hours) was much longer than that of the suspension (0.483 hours), indicating that the microemulsion drug delivery system was able to prolong the effective therapeutic time significantly. The CL/F of the microemulsion (1606.179 L/hour/kg) was a lot smaller than that of the suspension (10,124.586 L/hour/kg), indicating that drug contained in the suspension could be cleared rapidly in vivo. The $T_{\text{max}}$ of the suspension (0.33 hours) was shorter than that of the microemulsion (one hour). $T_{\text{max}}$ is generally believed to be responsible for drug release, and this phenomenon could explain why drug release from the microemulsion was slower than that from the suspension. After administration of the suspension, 5F underwent rapid distribution and penetration in tissues; however, in the case of a microemulsion, the drug is enwrapped in the inner phase, which would delay penetration

**Table 2 Pharmacokinetic parameters for 5F suspension and 5F microemulsion after oral administration to mice (n = 5)**

| Parameters | Unit       | 5F suspension | 5F microemulsion |
|------------|------------|---------------|------------------|
| AUC$_{\text{(0-t)}}$ | µg/hour | 1.955 ± 0.084 | 12.054 ± 0.656 |
| AUC$_{\text{(0-∞)}}$ | µg/L/hour | 1.975 ± 0.122 | 12.452 ± 1.066 |
| MRT$_{\text{(0-t)}}$ | Hours | 0.483 ± 0.036 | 2.451 ± 0.153 |
| MRT$_{\text{(0-∞)}}$ | Hours | 0.503 ± 0.055 | 2.698 ± 0.165 |
| CL/F | L/hour/kg | 10,124.586 | 1606.179 |
| $C_{\text{max}}$ | µg/L | 3.1776 ± 0.262 | 3.1221 ± 0.124 |
| $T_{\text{max}}$ | Hours | 0.33 ± 0.08 | 1.02 ± 0.16 |
| Relative bioavailability (%) | – | – | 616.57 |

**Abbreviations:** AUC, area under the concentration-time curve; 5F, ent-11α-hydroxy-15-oxo-kaur-16-en-19-oic acid; $C_{\text{max}}$, peak plasma concentration reached; $T_{\text{max}}$, time taken to reach peak plasma concentration; MRT, mean residence time; CL/F, apparent clearance.
and distribution into tissues, and this is consistent with our drug release tests shown in Figure 5.

It was evident that the relative bioavailability of the microemulsion versus the suspension was 616.57%,
despite there being little difference in drug release in vitro between the two delivery systems. What could explain this phenomenon and which was the critical factor influencing drug behavior in vivo?

It is well known that release and absorption are two important factors to be considered when evaluating the in vivo behavior of a drug substance. Our results indicate that drug absorption was a more critical factor than drug release. One unique property of a microemulsion is that, when loaded with a lipophilic drug, it has better absorption in the gastrointestinal tract, whereas when a microemulsion contains a poorly water-soluble drug, absorption is often inadequate because of insufficient dissolution in the gastrointestinal tract. In this study, the improved absorption of 5F from the formulation containing 1,2-propanediol and PEG-400 could reflect enhanced solubility. A relatively high ratio of surfactant in a microemulsion might also contribute to increased permeability by disturbing the cell membrane. In addition, the small droplet size (in the range of 50 nm) in a microemulsion may enable it to penetrate the absorption site via the transcellular pathway, and could protect the drug from enzyme degradation. Further, the higher AUC of the 5F microemulsion might result from enhanced absorption in the lymphatic pathway, given that the microemulsion is a lipid-based formulation. That would reduce the opportunity for hepatic first-pass metabolism and therefore enhance drug bioavailability. Our data show that the microemulsion was able to enhance drug bioavailability, which would improve therapeutic efficiency and have good potential for clinical application.

**In vivo toxicity studies**

**Biochemical markers of liver injury**

Hepatotoxicity was investigated by quantitative analysis of glutamic oxaloacetic transaminase activity, as shown in Table 3. The activity of this biochemical marker was approximately the same in the 5F suspension and control groups, indicating that 5F was not hepatotoxic, which is in accordance with our previous study in which all mice injected intraperitoneally with 5F suspension appeared to be healthy, with no liver or renal damage, although they were observed to have a mild degree of hair loss.

**Histological staining of liver tissue**

At necropsy, the gross appearance of the liver tissue was smooth and normal in color for the control, 5F suspension, and 5F microemulsion groups. However, the livers of mice treated with free triptolide appeared to be hyperemic and mottled (Figure 7) as well as fragile. The livers of mice treated with the 5F microemulsion and 5F suspension did not show any significant microscopic changes. There was also no significant difference in liver weight between either of the 5F formulation groups and the control group, indicating that neither 5F formulation was hepatotoxic to mice.

**Conclusion**

A microemulsion containing 5F, a poorly water-soluble drug, was formulated for oral delivery. The components and ratio ranges for the microemulsion were obtained from a solubility study, construction of a pseudoternary phase diagram, and analysis of droplet size. The optimum microemulsion formulation consisted of 45% water, 10% castor oil as the oil phase, 15% Cremorphor EL as the surfactant, and 30% as a cosurfactant mixture of 1,2-propanediol and PEG-400 (2:1), and achieved sufficient drug loading with a droplet size of less than 50 nm. In vitro, the release of 5F from the microemulsion

**Table 3 Results of blood biochemistry for glutamic oxaloacetic transaminase**

| Group (n = 5)          | 30 minutes | 4 hours | 24 hours |
|------------------------|------------|---------|----------|
| 5F suspension group    | 25.3 ± 3.62| 23.4 ± 3.16| 22.6 ± 4.82|
| 5F microemulsion group | 22.9 ± 4.82| 21.2 ± 2.88| 19.1 ± 4.38|
| Saline group           | 23.2 ± 3.64| 22.8 ± 4.22| 23.2 ± 2.18|

Abbreviation: 5F, ent-11α-hydroxy-15-oxo-kaur-16-en-19-oic-acid.
was slightly greater than that of the suspension. In vivo, the microemulsion showed better absorption than did the suspension. The relative bioavailability of the microemulsion compared with that of the suspension was 616.57%, and the microemulsion formulation was not hepatotoxic in mice. These results suggest the potential for 5F microemulsion to be administered by the oral route in the clinical setting.

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Disclosure
The authors report no conflicts of interest in this work.

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