Journal of Functional Foods 81 (2021) 104443

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

A novel amorphous preparation improved curcumin bioavailability in healthy volunteers: A single-dose, double-blind, two-way crossover study

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ARTICLE INFO

Keywords:
Curcumin
Bioavailability
Amorphous
Theracurmin®
curcuRouge™

ABSTRACT

Curcumin derived from *Curcuma longa* has beneficial pharmacological effects. However, its bioavailability is very low. To improve its bioavailability, we developed a novel amorphous formulation of curcumin, called curcuRouge™. We investigated the bioavailability of curcuRouge™ and compared its efficiency with that of Theracurmin®. Male Sprague–Dawley rats were orally administered curcuRouge™ or Theracurmin® (10 mg/kg of curcumin). Based on the area under the plasma concentration-time curve, the bioavailability of curcuRouge™ was 3.7-fold higher than that of Theracurmin® in rats. We performed a single-dose, double-blind, two-way crossover study to compare the bioavailability of curcuRouge™ and Theracurmin® (90 mg of curcumin) in 12 volunteers (8 males, 4 females). The bioavailability of curcuRouge™ was 3.4-fold higher than that of Theracurmin®. curcuRouge™ achieved C_{max} in a shorter time than Theracurmin® in both experiments. These findings indicate that curcuRouge™, an amorphous formulation of curcumin, shows superior bioavailability to that of Theracurmin®.

1. Introduction

The morbidity and mortality of lifestyle-related diseases, including type 2 diabetes, cardiovascular diseases, and cancer, has increased in developed countries (Malakar et al., 2019; Raghavan et al., 2019). These diseases are caused by abnormalities in multiple signaling pathways due to environmental, genetic, and aging factors. Thus, it is difficult to prevent the development of such diseases by blocking only one of these signaling pathways, and targeting a single pathway among many pathways is not likely to be effective for the prevention and treatment of these diseases (Bordoloi, Roy, Monisha, Padmavathi, & Kunnumakkara, 2016; Koeberle & Werz, 2014). Recent studies have shown the effects of natural products which contribute to preventing the diseases associated with biological regulatory system abnormalities. Thus, natural compounds have potential to be used in the prevention and treatment of these diseases.

Curcumin is a natural polyphenol extracted from the rhizome of *Curcuma longa*. It has been used as a traditional medicine to treat cough, jaundice, cold, hepatic disorders, and inflammatory diseases in India and China (Ammon & Wahl, 1991). Recently, curcumin has received considerable attention owing to its multiple pharmacological activities, such as anti-inflammatory, antioxidant, anti-viral, anti-tumor, and cell protective effects (Deguchi, 2015; Farhood et al., 2018; Khan, Ullah, & Nabavi, 2019), and it is expected to be used as a supplement to prevent lifestyle-related diseases and skin disorders (Salehi et al., 2019; Vollono, Falconi, Gaziano, Iacovelli, Dika, Terracciano, Bianchi, & Campione,

Abbreviations: AUC, Area Under the plasma Concentration-time curve; C_{max}, maximum plasma Concentration; UMIN, University hospital Medical Information Network; QOL, Quality Of Life; ASD, Amorphous Solid Dispersion; XRD, X-ray diffraction; SD, Sprague-Dawley; HPLC-MS/MS, Liquid Chromatography / Mass Spectrometry; UHPLC, Ultra High Performance Liquid Chromatography; SD, Standard Deviation; BMI, Body-Mass Index; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; PR, Pulse Rate; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure.

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https://doi.org/10.1016/j.jff.2021.104443
Received 16 November 2020; Received in revised form 3 February 2021; Accepted 7 March 2021
Available online 7 April 2021

2019). Despite its beneficial effects and low toxicity in humans, the poor water solubility and extremely low bioavailability of curcumin limit its widespread application (Cheng et al., 2001; Lao et al., 2006). Lao et al. reported that the maximum plasma concentration (C_{max}) of curcumin was only 50.5 and 57.6 ng/mL after 10 g and 12 g curcumin intake, respectively, in humans (Lao et al., 2006). Even if curcumin is orally administered at a high dose, only a small quantity of curcumin is detected in the blood plasma. Moreover, curcumin in plasma is rapidly metabolized and excreted via faeces and urine (Niu et al., 2012).

To overcome the limitations caused by low curcumin bioavailability, many studies of curcumin delivery systems, such as particles, micelles, emulsions, and liposomes have been conducted (Chebl, Moussa, Peurla, & Patra, 2017; Feng, Wei, Lee, & Zhao, 2017; Guerrero et al., 2018; Tan et al., 2018). In our previous study, we prepared an effective curcumin preparation called Theracurmin®, which is a submicron-particle colloidal dispersion (Sasaki et al., 2011). Theracurmin® achieved a 27-fold higher area under the plasma concentration-time curve (*AUC*) at 0–6 h in humans compared with native curcumin. The C_{max} of Theracurmin® was 10.7- and 5.6-fold higher than that of the commercial curcumin preparations BCM-95® and Meriva®, respectively (Sunagawa et al., 2015).

In this study, we prepared a new amorphous solid dispersion of curcumin and named it curcuRouge™. We investigated the bioavailability of curcuRouge™ and compared it with that of Theracurmin® in rats and in humans by performing a randomized, double-blind, two-way crossover clinical study.

2. Materials and methods

2.1. Chemicals

Native curcumin powder (98% pure, CAS#458-37-7), mepronil (98% pure, CAS#55814-41-0), acetonitrile (99.6% pure, CAS#75-05-8), methanol (99.7% pure, CAS#67-56-1), formic acid (98% pure, CAS#64-18-6), sodium acetate (98.5% pure, CAS#127-09-3), chloroform (99% pure, CAS# 67-66-3), and β-glucuronidase (100,000 units per mL, CAS#9001-45-0) were purchased from FUJIFILM Wako Chemicals (Osaka, Japan). Theracurmin® was purchased from the market. Theracurmin® consisted of 10w/w% curcumin and other components (Table 1) (Sasaki et al., 2011). One capsule of Theracurmin® contains 30 mg of curcumin.

Table 1
Composition of each curcumin capsule.

Sample	Manufacturing method	Ingredients	Curcumin content per capsule	Curcumin content per formulation
curcuRouge™	Amorphous formulation of curcumin	*Curcuma longa* extract, Modified starch, Cornstarch	30 mg	37w/w%
Theracurmin®	Curcumin dispersed with colloidal submicron-particles	Theracurmin (Dextrin, Maltose, *Curcuma longa* extract, Gum ghatti, Citric acid), Cornstarch, Silicon dioxide, Calcium stearate	30 mg	10w/w%

2.2. Preparation of curcuRouge™

curcuRouge™ was prepared and supplied by Therabiopharma Inc. Briefly, native curcumin was flattened on an aluminum dish and melted at 220 °C using hot plate. After cooling to room temperature, the sample was coarsely ground using a dry-impact type pulverizer. This sample was mixed with modified starch, ground in distilled water using a continuous horizontal-type ready mill RMH-03 (Aimex co Ltd. Tokyo, Japan), and freeze-dried, resulting in a stable fine powder of curcuRouge™. curcuRouge™ consisted of 37.3w/w% of curcumin and other components, as shown in Table 1. X-ray diffraction (XRD) analysis was performed using a MiniFlex diffractometer (Rigaku Co., Japan). Microscopic images were taken using a Primovert inverted microscope (Zeiss, Deutschland). Native curcumin, Theracurmin®, and curcuRouge™ (containing 4.5 mg of curcumin, respectively) were dispersed in 3 mL of distilled water and observed at 0, 1, 24, and 96 h after vortex-mixing.

2.3. Experimental design in rats

The animal experiment conformed to the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animals, University of Shizuoka (US#176279). Rats were housed in pathogen-free conditions, received food and water ad libitum, and were maintained in a 12 h dark/light cycle in an appropriate temperature- (19.5–29.4 °C) and moisture- (34.5–75.4%) controlled room.

The experiment was performed according to a previous study (Sunagawa et al., 2012). Briefly, 10 male Sprague–Dawley rats (8-week-old; 250–290 g) were randomly assigned to the Theracurmin® (N = 5) and curcuRouge™ (N = 5) groups and fasted for 12 h before administration. Theracurmin® and curcuRouge™ were directly suspended in distilled water. The final content of curcumin was 0.25% in both solutions. The rats were orally administrated an alternative solution at a dosage of 10 mg curcumin/kg body weight by oral gavage. Blood samples were collected from the tail of rats at 0, 0.25, 0.5, 1, 2, 4, and 6 h after administration, placed in dark-colored heparinized tubes to protect from light, and centrifuged at 1,500*g* for 10 min. Next, 0.1 mL of the supernatant was collected in dark-colored tubes and stored at −20 °C until analysis.

2.4. Clinical study in humans

The clinical study was approved by the Clinical Research Ethics Committees of Shizuoka General Hospital (SGHIRB#2019071), University of Shizuoka (IRB#1-25), and Kyoto Medical Center (IRB#19-056). The study was conducted in accordance with the ethical principles based on the Helsinki Declaration, and it was registered with the University hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN000039083). This single-dose, double-blind, two-way crossover study was performed from 8 January 2020 to 2 February 2020 at Shizuoka General Hospital and the University of Shizuoka in Japan. All volunteers provided written informed consent prior to participation in compliance with the Declaration of Helsinki. The inclusion criteria is healthy adults aged 19–60 years old with a body mass index (BMI) of 18.0–30.0 kg/m^2 at screening. The exclusion criteria included the use of medicines or dietary supplements containing curcumin, pregnancy, lactation, chronic diseases, and smoking. The screening procedures included medical history examination, physical examination, hematologic profiling, and biochemistry analysis.

For the clinical study, curcuRouge™ (30 mg curcumin) was contained in a hard capsule. Theracurmin® (30 mg curcumin in a capsule) and curcuRouge™ were labeled; however, the volunteers and researchers were blinded to the labeling. The clinical study was performed according to a previous study (Morimoto et al., 2013). The participants were divided into two groups. The volunteers did not consume curcumin-containing food for more than one week before this study. On the day before the study, they finished dinner by 9 PM and fasted

overnight (water intake was not restricted). The next morning, blood pressure was measured. After that, the volunteers consumed the curcumin preparations (3 capsules, 90 mg curcumin) with a sip of mineral water. Blood specimens were collected immediately before and at 0.5, 1, 2, 4, and 8 h after taking the preparations. After blood sampling at 4 h, the volunteers were fed a rice ball containing pickled plum for lunch. All blood specimens were collected in 5-mL blood-collecting vessels containing heparin and immediately centrifuged at 1,500*g* for 10 min. Next, 0.5 mL of the supernatants was collected in dark-colored tubes and stored at −20 °C until analysis. After a washout period of 1–2 weeks, the alternate curcumin preparation was administered using the same protocol. After measurement of the total curcumin concentration in plasma, these data were fixed, and the labels were opened. Biochemical parameters, including total cholesterol, triglyceride, creatinine, urine albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (γGTP) were analyzed by Hoken Kagaku, Inc. (Shizuoka, Japan).

2.5. Power analysis

Power analysis was performed using the G*Power 3.1.9.2 software (Faul, Erdfelder, Lang, & Buchner, 2007). A previous crossover study examined the effects of Theracurmin® in healthy volunteers (dropout rate: 0%) (Morimoto et al., 2013; Sunagawa et al., 2015). Based on the result of *AUC* values, power analysis was performed with an effect size of 0.5, power of 0.7, and significance level of 0.05. Assuming a dropout rate of 5%, the sample size for the current study was determined to be twelve volunteers.

2.6. Sample preparation and measurement of plasma curcumin levels

HPLC-MS/MS system comprising the Elute UHPLC system (Bruker Japan, Japan) and micrOTOF compact (Bruker Japan, Japan) with (+) electrospray ionization was used for measuring curcumin concentration. The samples were separated using a Sunshell C-18 column (2.1 × 100 mm, 2.6 μm, ChromaNik Technologies Inc., Osaka, Japan) and a gradient of binding solvent (0.1% formic acid/H_2O) and elution solution (0.1% formic acid/acetonitrile) at a flow rate of 0.4 mL/min, and column temperature of 40 °C. The separation was conducted by liner gradient (5–95% elution solvent). The mass spectrometer was operated under multiple reaction monitoring with electron spray thermo ionization. The transitions (precursor to product) monitored were *m/z* 369.1 → 177.2 (*m/z*) for curcumin, and 270 → 119 (*m/z*) for mepronil.

Plasma curcumin levels were measured as reported previously (Sasaki et al., 2011). Briefly, 0.1 mL of plasma sample was transferred to a 10 mM glass tube, to which 0.11 mL of 0.1 M sodium acetate buffer (pH 5.0) containing 1,000 U β-glucuronidase was added. The mixture was then allowed to react at 37 °C for 1 h to hydrolyze the curcumin conjugates, and then 10 μL of internal standard solution (500 ng/mL mepronil) and 0.5 mL chloroform were added. The sample was vortexed for 1 min and centrifuged at 1,500 × *g* for 5 min. The organic layer was transferred to a 1-mL tube and evaporated to dryness using a centrifuge concentrator. The dried sample was redissolved in 100 μL of 50% acetonitrile containing 0.05% formic acid and centrifugated at 7,700*g* for 5 min. A 10 μL of supernatant was injected into the HPLC-MS/MS system. The total curcumin concentration (free and conjugated curcumin) in plasma was calculated.

2.7. Pharmacokinetics

The *AUC* was calculated using the trapezoidal method (Sasaki et al., 2011). The C_{max} and time to reach maximum concentration (T_{max}) were obtained directly from the measured data. The relative values among native curcumin, Theracurmin®, and curcuRouge^{TM} were calculated using individual data, and the mean values were then represented.

2.8. Statistical analysis

JMP Software version 12.2 (SAS Institute Inc.) was used for statical analysis. Data are expressed as the mean ± standard deviation (S.D.). For evaluation of plasma curcumin concentration and pharmacokinetic parameters in rats and healthy volunteers, a non-parametric Mann-Whitney *U* test was performed. Sex differences were compared by one-way ANOVA. $p < 0.05$ was considered significant.

3. Results

3.1. XRD analysis and microscope images of curcuRouge^{TM}

The physical formulation of curcuRouge™ and native curcumin were characterized using the XRD system. As shown in Fig. 1, the XRD pattern of native curcumin showed several intense peaks, which indicated the crystal forms of curcumin. In contrast, the XRD pattern of curcuRouge^{TM} showed a typical halo form without characteristic peaks. Microscopic images of curcuRouge™, Theracurmin®, and native curcumin are shown in Fig. 2A. Theracurmin® and native curcumin are yellow in color, whereas curcuRouge^{TM} has a light red shade. Native curcumin showed crystal piece aggregates of various sizes, whereas Theracurmin® showed very small, uniform particles (0.19 μm) (Sasaki et al., 2011). The particle size of curcuRouge^{TM} (<10 μm) was non-uniform and larger than that of Theracurmin®. The dispersion stability of curcumin preparations in distilled solution is shown in Fig. 2B. Theracurmin® maintained a dispersed state even at 96 h after vortex-mixing, whereas native curcumin showed crystal precipitation at 1 h after vortex-mixing. curcuRouge^{TM} showed precipitation at 24 h after vortex-mixing. The upper part of the solution was clear at 96 h.

3.2. Bioavailability of curcuRouge^{TM} and Theracurmin® in rats

The total curcumin concentrations in plasma at 0, 0.25, 0.5, 1, 2, 4, and 6 h are shown in Fig. 3. The total curcumin concentration was significantly higher until 4 h in rats administered curcuRouge^{TM} than in those administered Theracurmin®. The pharmacokinetic parameters, including *AUC*, C_{max}, and T_{max} are presented in Table 2. The $AUC_{0\text{-}6h}$ of curcuRouge™ (2956 ± 933 nmol/L·h, $p < 0.001$) was significantly higher than that of Theracurmin® (805 ± 171 nmol/L·h). The C_{max} values of curcuRouge^{TM} and Theracurmin® were 2634 ± 378 and 274 ± 48 nmol/L, respectively ($p < 0.001$). The T_{max} value of curcuRouge^{TM} (0.25 ± 0.00 h, $p < 0.004$) was significantly shorter than that of Theracurmin® (0.60 ± 0.20 h). These results indicated that the bioavailability of curcuRouge^{TM} was 3.7-fold higher than that of Theracurmin®. Moreover, curcuRouge™ achieved C_{max} in a shorter time and its C_{max} was 9.6-fold higher than that of Theracurmin®.

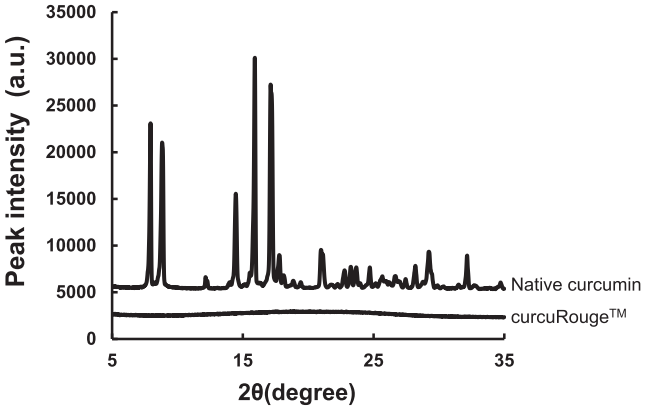
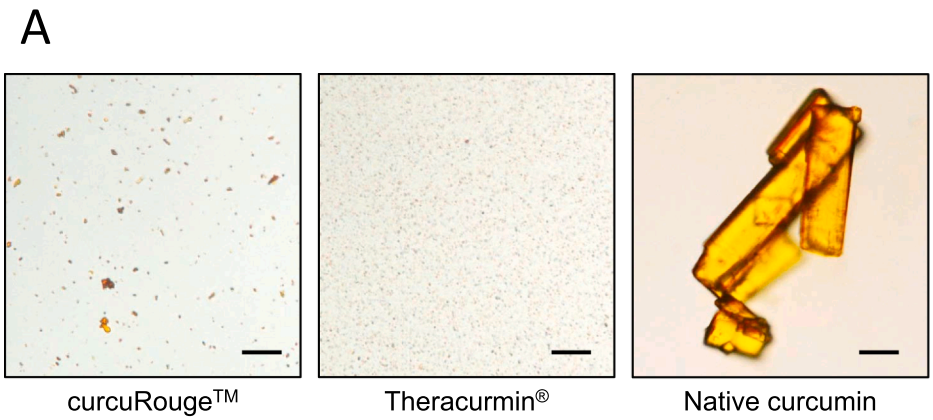


Fig. 1. X-ray diffraction patterns of curcuRouge™ and native curcumin.



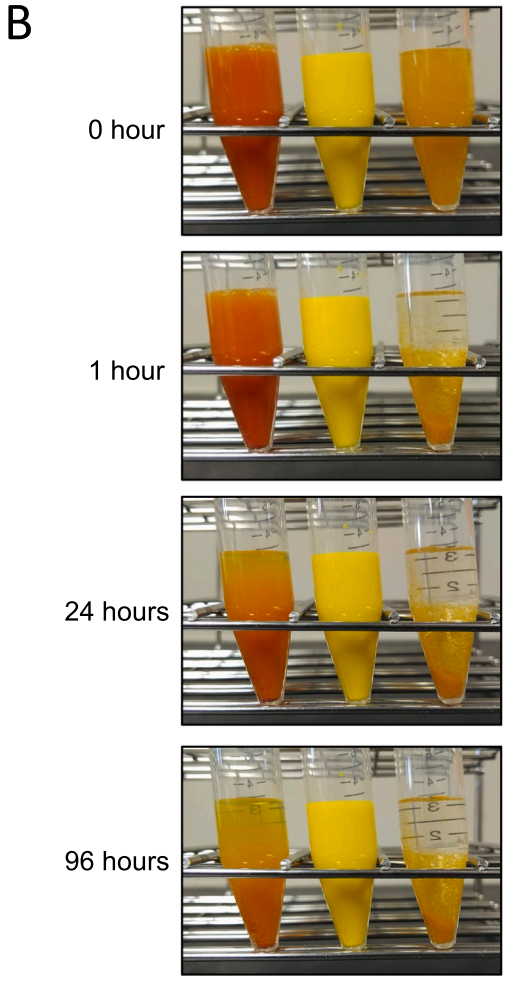


Fig. 2. Microscopic images and dispersion stability of curcuRouge™, Theracurmin®, and native curcumin. *A*, Photographic images of curcuRouge™ (*left*), Theracurmin® (*center*), and native curcumin (*right*). CurcuRouge™, Theracurmin®, and native curcumin were dispersed in distilled water (the final content of curcumin, 1.5 mg/mL) and observed by using the optical microscope. 400× magnification. The scale bar is 20 μm. *B*, CurcuRouge™ (*left*), Theracurmin® (*center*), and native curcumin (*right*) were dispersed in 3 mL of distilled water (final content of curcumin: 1.5 mg/mL). After vortex-mixing for 10 s, these were observed at 0, 1, 24, and 96 h.

3.3. Volunteer characteristics and disposition

Twelve healthy volunteers (eight males and four females) were enrolled in this study; their mean age was 23.0 ± 2.6 years (male) and 22.3 ± 0.5 years (female). The results of hemodynamic and biochemical analyses are presented in Table 3. The liver and kidney function parameters of all volunteers were within the reference ranges. No volunteer discontinued the study, and no adverse effects were observed during this study.

3.4. Bioavailability of curcuRouge™ and Theracurmin® in humans

The plasma curcumin concentrations at 0, 0.5, 1, 2, 4, and 8 h are shown in Fig. 4. curcuRouge™ exhibited significantly higher plasma curcumin concentrations at 0.5, 1, 2, and 4 h than Theracurmin®. The pharmacokinetic parameters are presented in Table 4. The $AUC_{0\text{–}8h}$ of curcuRouge™ (4214 ± 2574 nmol/L·h, $p < 0.001$) was 3.4-fold higher than those of Theracurmin® (1243 ± 779 nmol/L·h, respectively). The C_{max} values of curcumin were 2.0 ± 1.5 h after intake of curcuRouge™ and 5.3 ± 3.1 h after administration of Theracurmin® ($p > 0.012$). The C_{max} of curcuRouge™ (1337 ± 955 nmol/L, $p < 0.002$) was 5.4-fold higher than that of Theracurmin® (248 ± 141 nmol/L). These results

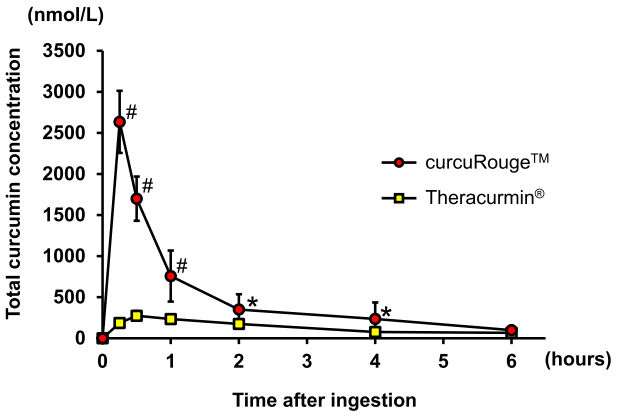


Fig. 3. Changes in plasma curcumin concentrations in rats. SD rats were orally administrated with curcuRouge™ and Theracurmin® (curcumin 10 mg/kg body weight). The total curcumin concentrations in plasma at 0, 0.5, 1, 2, 4, and 6 h were measured and graphically represented. Red circle: curcuRouge™; Yellow square: Theracurmin®. Each point and bar represent the mean ± standard deviation (S.D.) (n = 5). Statistical differences between curcuRouge™ and Theracurmin® group were analyzed by non-parametric Mann-Whitney *U* test. *:$p < 0.05$, #:$p < 0.01$.

indicated that the bioavailability of curcuRouge™ significantly improved compared with that of Theracurmin® and could achieve the T_{max} in a shorter time than Theracurmin® in humans.

4. Discussion

In this study, we developed curcuRouge™, an amorphous curcumin preparation, to improve the bioavailability of curcumin. Oral absorption experiments in rats showed that the C_{max} and AUC_{0-6h} of curcuRouge™ were 9.6- and 3.7-fold higher than those of Theracurmin®, respectively. The clinical study revealed that the C_{max} and AUC_{0-8h} of curcuRouge™ were 5.4- and 3.4-fold higher than those of Theracurmin®, respectively. In both experiments, we confirmed that curcuRouge™ could achieve the T_{max} in a shorter time than Theracurmin®. These results demonstrated that the novel curcumin preparation, curcuRouge™ exhibited more than 3-fold higher bioavailability than Theracurmin®.

Curcumin is expected to be applied for the prevention of various diseases, such as cardiovascular diseases, cancer, and inflammatory diseases (Morimoto et al., 2018; Salehi et al., 2019; Sunagawa, Katanasaka, Hasegawa, & Morimoto, 2015; Vollono et al., 2019). However, curcumin concentration in plasma was only detectable after the administration of native curcumin at gram dosages (Cheng et al., 2001; Lao et al., 2006). The low bioavailability of curcumin inhibits its approval as a therapeutic agent. To improve the bioavailability of curcumin, we successfully prepared the novel formulation of curcumin, named curcuRouge™. Generally, the bioavailability and stability of poorly water-soluble drugs is improved in their amorphous forms (Tran, Duan, Lee, & Tran, 2019). XRD analysis revealed that the waveform pattern of curcuRouge™ was a broad peak (Fig. 1) whereas that of native curcumin showed several intense peaks. The XRD patterns of Theracurmin® and native curcumin were the same. The production of an amorphous formulation is not possible in the manufacturing of Theracurmin®, which involves ground milling and dispersion by a high-pressure homogenizer (Sasaki et al., 2011). Native curcumin is brilliant yellow at pH 2.5 to 7 and turns red at pH 7. (Kunnumakkara, Anand, & Aggarwal, 2008). In contrast to native curcumin and Theracurmin®, curcuRouge™ had a light red shade because of being manufactured under weak alkali conditions. The particle size of curcuRouge™ was non-uniform and below 10 μm (Fig. 2A). Theracurmin® had uniform particles (0.19 μm) (Sasaki et al., 2011) because of micronization using a wet grinding mill. Native curcumin showed aggregation property in

Table 3
Baseline characteristics of the subjects (healthy male and female).

	Male (n = 8)	Female (n = 4)	P value
Age (year)	23.0 ± 2.6	22.3 ± 0.5	0.45
BMI (kg/m^2)	21.2 ± 2.4	20.9 ± 2.0	0.23
SBP (mmHg)	119 ± 12	105 ± 10	0.09
DBP (mmHg)	70 ± 9	67 ± 8	0.58
PR (bpm)	64 ± 7	64 ± 10	0.64
Total cholesterol (mg/dL)	173 ± 21	179 ± 29	0.73
Triglyceride (mg/dL)	60 ± 20	72 ± 36	0.56
Creatinine (mg/dL)	0.82 ± 0.09	0.64 ± 0.15	0.09
Urine albumin (g/dL)	4.8 ± 0.3	4.9 ± 0.5	0.58
AST (IU/L)	19 ± 2	21 ± 1	0.26
ALT (IU/L)	18 ± 3	14 ± 4	0.13
γGTP (IU/L)	19 ± 4	18 ± 9	0.83

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, PR: pulse rate, AST: aspartate aminotransferase, ALT: alanine aminotransferase, γGTP: gamma-glutamyl transpeptidase. Sex difference was compared by one-way ANOVA. Mean values ± Standard Deviation.

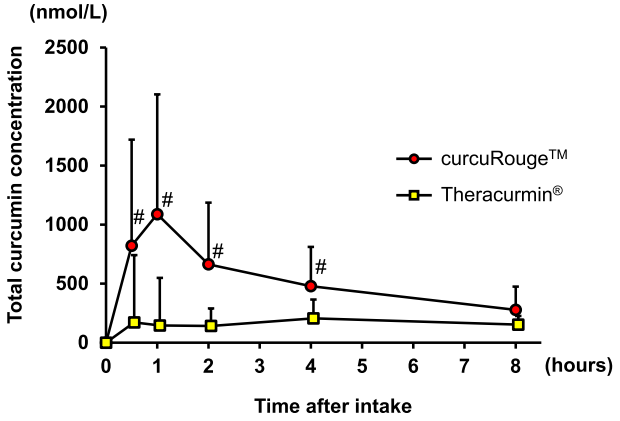


Fig. 4. Changes in plasma curcumin concentrations in twelve healthy volunteers. Twelve healthy volunteers (8 males and 4 females) were orally administrated with curcuRouge™ and Theracurmin® containing 90 mg of curcumin in a double-blind 2-way crossover study. The total curcumin concentrations in plasma at 0, 0.5, 1, 2, 4, and 8 h were measured and graphically presented. Red circle: curcuRouge™; Yellow square: Theracurmin®. Each point and bar represent the mean ± standard deviation (S.D.) (n = 12). Statistical differences between curcuRouge™ and Theracurmin® group were analyzed by non-parametric Mann-Whitney *U* test. *:$p < 0.05$, #:$p < 0.01$.

Table 2
Pharmacokinetic parameters of total curcumin concentration in rats after an oral administration with curcuRouge™ and Theracurmin® (curcumin 10 mg/kg body weight).

	CurcuRouge™ (n = 5)	Theracurmin® (n = 5)	Relative value (CurcuRouge™ versus Theracurmin®)	P value
AUC_{0-6h} (nmol/L·h)	2956 ± 933	805 ± 171	3.7-fold	0.001
C_{max} (nmol/L)	2634 ± 378	274 ± 48	9.6-fold	0.001
T_{max} (h)	0.25 ± 0.00	0.60 ± 0.20	0.4-fold	0.004

AUC: area under the plasma concentration time curve, C_{max}, maximum plasma concentration, T_{max}: time to reach maximum concentration. For evaluation of pharmacokinetic parameters in rats, non-parametric Mann-Whitney *U* test was performed. Mean values ± Standard Deviation.

Table 4
Pharmacokinetic parameters of total curcumin concentration of healthy volunteers with an oral administration of curcuRouge™ and Theracurmin® (curcumin 90 mg) in double-blind 2-way crossover study.

	CurcuRouge™ (n = 12)	Theracurmin® (n = 12)	Relative values (curcuRouge™ versus Theracurmin®)	P value
$AUC_{0\text{-}1h}$ (nmol/L·h)	682 ± 668#	45 ± 31	15.2-fold	0.002
$AUC_{0\text{-}2h}$ (nmol/L·h)	1558 ± 1372#	178 ± 100	8.8-fold	0.001
$AUC_{0\text{-}4h}$ (nmol/L·h)	2701 ± 1930#	485 ± 314	5.6-fold	0.001
$AUC_{0\text{-}8h}$ (nmol/L·h)	4214 ± 2574#	1243 ± 779	3.4-fold	0.001
C_{max} (nmol/L)	1337 ± 955#	248 ± 141	5.4-fold	0.002
T_{max} (h)	2.0 ± 1.5*	5.3 ± 3.1	0.4-fold	0.012

AUC: area under the plasma concentration time curve, C_{max}, maximum plasma concentration, T_{max}: time to reach maximum concentration. For evaluation of pharmacokinetic parameters in rats, non-parametric Mann-Whitney *U* test was performed. Mean values ± Standard Deviation

water. Theracurmin® maintained high dispersion stability for up to 96 h in aqueous solution. The precipitation of curcuRouge™ was clearly shown at the same time owing its larger particle size than that of Theracurmin® (Fig. 2B). These findings indicated that curcuRouge™ was an amorphous formulation of curcumin compared with those of Theracurmin® and native curcumin.

It is clear that native curcumin has extremely low bioavailability in humans (Cheng et al., 2001; Lao et al., 2006). Native curcumin almost did not show any dose-dependency. A comparative study between a high-dose curcumin preparation and native curcumin may reveal the higher bioavailability of curcumin. Thus, it is difficult to evaluate the bioavailability of curcumin preparations by using native curcumin as a control. One of the countermeasures for this problem is utilization of existing curcumin preparations, such as Theracurmin®, Meriva®, and BCM-95®. These preparations are commercially available in many countries and have a better index than native curcumin as their bioavailability has been well investigated (Antony et al., 2008; Cuomo et al., 2011; Sasaki et al., 2011). In this study, we performed a comparative study in rats and humans and found that curcuRouge™ had > 3-fold higher bioavailability than Theracurmin®. The novel amorphous formulation of curcuRouge™ is considered to contribute to improving the bioavailability of curcumin.

Currently, several curcumin preparations with high absorption are commercially available. The bioavailability data of different formulations are summarized in Table 5. Meriva® is a mixture of curcumin and phosphatidylcholine derived from soybean lecithin and contains 20% curcuminoids (Cuomo et al., 2011). It has been shown to have 29 times higher absorption than native curcumin. Longvida® is also a solid lipid curcumin particle-based formulation with soy lecithin containing purified phospholipids (Gota et al., 2010). A comparative pharmacokinetic study in healthy volunteers demonstrated that the bioavailability of Longvida® is 100 times higher than that of curcuminoids. BCM-95® is a

Table 5
Comparison of curcumin bioavailability between different commercially available preparations in human study.

	Formulation of curcumin	Subjects	Used dosage of curcumin or curcuminoids	*AUC* (nmol/L·h)	*AUC* per dosage (nmol/L·h/mg)	References
CurcuRouge™	Amorphous formulation of curcumin	Healthy subjects (n = 12)	90 mg curcumin	4214[a]	46.8	In this study
Theracurmin®	Colloidal submicron dispersion of curcumin	Healthy subjects (n = 12)	90 mg curcumin	1243[a]	13.8	In this study
		Healthy subjects (n = 7)	30 mg curcumin	306[b]	10.2	Sasaki et al., 2011
		Healthy subjects (n = 9)	180 mg curcumin	2329[b]	12.9	Sunagawa et al., 2015
Meriva®	A mixture of curcumin and phosphatidylcholine derived from soybean lecithin	Healthy subjects (n = 9)	297 mg curcumin	1457[c]	4.9	Cuomo et al., 2011
		Healthy subjects (n = 12)	150 mg curcumin	476[b]	3.2	Sunagawa et al., 2015
BCM95®	A mixture of curcumin and turmeric-derived essential oils	Healthy subjects (n = 11)	1300 mg curcumin	8677[d]	6.7	Antony et al., 2008
		Healthy subjects (n = 12)	260 mg curcumin	202[b]	0.8	Sunagawa et al., 2015
Longavida®	A solid lipid curcumin particle-based formulation with soy lecithin	Healthy subjects (n = 6)	130–195 mg curcumin	484[d]	2.5–3.7	Gota et al., 2010
CurcuWin®	A water-soluble formulation of curcumin comprising polyvinyl pyrrolidine	Healthy subjects (n = 12)	376 mg curcuminoids	834[e]	2.2	Jäger et al., 2014
NovaSol®	A micelle formulation of curcumin with Tween-80	Healthy subjects (n = 23)	410 mg curcumin	12147[c]	29.6	Schiborr et al., 2014
		Healthy subjects (n = 23)	80 mg curcumin	574[c]	7.2	Kocher, Schiorr, Behnam, & Frank, 2015

AUC: area under the plasma concentration time curve.
a: $AUC_{0\text{-}8h}$.
b: $AUC_{0\text{-}6h}$.
c: $AUC_{0\text{-}24h}$.
d: $AUC_{0\text{-}infinity}$.

mixture of curcumin and turmeric-derived essential oils, and its bioavailability has been shown to be 6.9 times higher than that of native curcumin (Antony et al., 2008). CurcuWin® is a water-soluble formulation comprising polyvinyl pyrrolidine as a hydrophilic carrier, cellulosic derivatives, and natural antioxidants (Jäger et al., 2014). The relative absorption of curcumin in CurcuWin® is 136 times higher than that of native curcumin. NovaSol® is a micelle formulation with Tween-80 as a nonionic surfactant (Schiborr et al., 2014; Kocher et al., 2015). Its bioavailability is 185 times higher than that of native curcumin. These preparations have shown superior bioavailability to that of native curcumin in humans. However, because the baseline characteristics of the subjects, the dosage of curcumin, and blood sampling time points were different between, it is not possible to directly compare these bioavailability values. Nevertheless, we calculated the *AUC* values per dose of curcumin or curcuminoids and showed that curcuRouge™ had the highest value among curcumin formulations. These data indicated that curcuRouge™ is a curcumin preparation with extremely high bioavailability in humans.

5. Conclusion

The novel curcumin preparation with amorphous formulation, curcuRouge™ showed > 3-fold higher bioavailability than that of Theracurmin® in rats and humans. Therefore, this novel type of curcumin preparation may be safe and useful to enhance the physiological activities of curcumin at low doses.

Authors' contributions

Yoichi Sunagawa and Tatsuya Morimoto designed this study. Yusuke Miyazaki, Masafumi Funamoto, Kana Shimizu, and Satoshi Shimizu conducted clinical research. Koji Hasegawa provided essential advice for clinical research. Nurmila Sari and Yasufumi Katanasaka analyzed the data and performed statistical analysis. Masashi Ito, Tatsuya Ogawa, and Hitomi Ozawa-Umeta performed the animal experiment, measured plasma curcumin levels, and provided curcuRouge™. Yoichi Sunagawa, Koji Hasegawa, and Tatsuya Morimoto wrote the manuscript. Tatsuya Morimoto had the primary responsibility for determining the final content. All authors have read and approved the final manuscript.

Ethics Statements

The animal experiment conformed to the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animals, University of Shizuoka (US#176279).

The clinical study was approved by the Clinical Research Ethics Committees of Shizuoka General Hospital (SGHIRB#2019071), University of Shizuoka (IRB#1-25), and Kyoto Medical Center (IRB#19-056). The study was conducted in accordance with the ethical principles based on the Helsinki Declaration, and it was registered with the UMIN Clinical Trials Registry (UMIN000039083). This single-dose, double-blind, two-way crossover study was performed from 8 January 2020 to 2 February 2020 at Shizuoka General Hospital and the University of Shizuoka in Japan. All volunteers provided written informed consent prior to participation in compliance with the Declaration of Helsinki.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Yusaku Matsushita, Mizuki Unno, Kanako Tsuchitani, Hiroko Mochizuki, Yuko Iwasaki, and Eriko Morimoto for their technical assistance. We would like to thank Editage (www.editage.com) for English language editing.

Funding

This work was supported by a grant from the Japan Science and Technology Agency (T. Morimoto; 17K08279, Hasegawa; 18K08121) and joint research funding from Therabiopharma Inc.

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