Drinkable Preparation of Theracurmin Exhibits High Absorption Efficiency—A Single-Dose, Double-Blind, 4-Way Crossover Study

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Received February 18, 2013; accepted August 25, 2013

Curcumin has various biological activities including antioxidant and antiinflammatory actions, and alcohol detoxification. However, because of its poor absorption efficiency, it is difficult for orally administered curcumin to reach blood levels sufficient to realize its bioactivities. We have generated capsules and tablets containing Theracurmin, a highly absorptive curcumin. In addition, we recently created a drinkable preparation of Theracurmin. To evaluate the absorption efficiency of this type of curcumin, we performed a single-dose, double-blind, 4-way crossover study. We compared plasma curcumin levels after the administration of Theracurmin beverage and 3 other drinkable types of curcumin sold in Japan. Twenty-four healthy subjects (male/female=13/11, age: 23–32) were administered with these 4 drinkable preparations of curcumin. The area under the blood concentration–time curve at 0–8 h was found to be 1.5 to 4.0-fold higher with Theracurmin than with the other 3 kinds of curcumin beverage. Moreover, maximal plasma curcumin concentrations (0–8 h) of Theracurmin were 1.8 to 3.8 times higher than those of the other 3 curcumin beverages. These data indicate that our newly prepared Theracurmin beverage exhibits a much better absorption efficiency than other kinds of curcumin beverage sold in Japan.

Key words curcumin; drinkable preparation; Theracurmin; human; plasma level

Curcumin is a yellow-colored substance widely known as turmeric, which is prepared from the root of the *Curcuma longa* plant, a member of the ginger family (Zingiberaceae) and native to India and Southeast Asia.[1)] Curcumin is well-known to have a broad spectrum of biological and pharmacological activities,[2)] such as antioxidant,[3–6)] antiinflammatory,[3,6,7)] antibacterial,[8)] antifungal,[9)] and anticarcinogenic activities,[10,11)] as described in many reports. Moreover, curcumin also possesses cardio- and neuroprotective effects.[12–14)] Curcumin has been used to treat a broad range of common ailments in Indian Ayurvedic medicine for at least 4000 years, as well as in Chinese, Arabic, and other traditional medicines. Curcumin is in modern use worldwide as a cooking spice, flavouring agent, and colorant. One of the reasons why curcumin has a broad and long history of use is its safety. No studies in either animals or humans have demonstrated significant toxicity associated with the use of curcumin, even at high doses.[15,16)] However, a problem with the use of curcumin is its poor water solubility and short biological half-life.[17,18)]

Several approaches have been tested to increase the oral bioavailability of curcumin, including adjuvant, nanoparticles, micelles, phospholipid delivery systems, and liposomes.[18–21)] However, no suitable delivery options, such as a soluble formulation of curcumin, have been found so far. Here, we have generated Theracurmin, a highly absorptive form of curcumin, using a technique of a micro-particle and surface-controlled colloidal dispersion. Theracurmin consisted of 10 w/w% of curcumin, 2% of other curcuminoids such as demethoxycurcumin and isdemethoxycurcumin, 46% glycerin, 4% gum ghatti, and 38% water. Theracurmin demonstrated oral bioavailability nearly 30-times higher than that of curcumin powder in both rats and humans.[22)] A minimal dose of Theracurmin was sufficient to improve the left ventricular systolic function in post-myocardial infarction rats, suggesting the clinical usefulness of Theracurmin for heart failure treatment.[23)]

Several curcumin beverages are sold as health foods in many countries including Japan. However, it has yet to be determined whether plasma curcumin levels sufficient to demonstrate bioactivities can be obtained by taking these beverages. Without improving the absorption efficiency of these beverages containing curcumin, their bioactivities might not be significant. We created the drinkable preparation of Theracurmin and performed a clinical study in humans using Theracurmin beverage and other curcumin beverages sold in Japan to compare the plasma levels of curcumin.

MATERIALS AND METHODS

Drinkable Preparations Theracurmin was obtained from Theravalues Corporation (Tokyo, Japan).[22)] A drinkable preparation containing Theracurmin was prepared. There other 3 commercial drinkable preparations containing curcumin were obtained from a store. These 4 drinkable preparations were re-bottled in new, unified bottles and labeled A to D, respectively. The contents of these health beverages are indicated in

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Table 1.

Subjects The Institutional Review Board at Shizuoka General Hospital approved this study. All subjects provided written informed consent prior to participation. Twenty-four healthy subjects consented to be in the study. Screening procedures included a medical history, physical exam, hematologic profile, and blood chemistries. Subjects were not taking any medications nor were they taking any dietary or herbal supplements. Women could not be pregnant or breast-feeding.

Study Design and Procedures Subjects participated in a single-dose, double-blind, 4-way crossover study. Subjects were divided into four groups as shown in Table 2. In each group, the four types of drinkable preparations of curcumin were administered every 7 d. Subjects did not take curcumin containing food for more than 7 d before this study and fasted overnight except for water. In the morning, blood specimens were obtained immediately prior to the drinkable preparations and at 0.5, 1, 2, 4, and 8 h after taking the drinkable preparations. Subjects received a light lunch 6 h after taking the preparations. All blood specimens were drawn in 5-mL blood-collecting vessels containing heparin. They were immediately placed in an ice bath and protected from the light. Vessels were centrifuged at 3000 RPM for 20 min at 4°C to separate the plasma. The plasma samples were then frozen at −70°C.

Sample Preparation and Measurement of Plasma Curcumin Levels Blood sample preparation and the measurement of plasma curcumin levels were previously reported.[22] Briefly, each plasma sample was incubated with 0.1 M sodium acetate buffer (pH 5.0) containing 1000 U β-glucuronidase (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at 37°C for 1 h to hydrolyze the curcumin conjugates. After extraction with chloroform, the dried extracts were reconstituted in 100 μL of 50% methanol and injected into a chromatographic system. Plasma concentrations of curcumin were measured using the HPLC-MS/MS system comprising the Prominence micro-LC system (Shimadzu, Kyoto, Japan) and an API 3200 tandem mass spectrometer (Applied Biosystems, CA, U.S.A.) with (+)electrospray ionization (ESI), as described previously.[23]

Pharmacokinetics The area under the curve (*AUC*) was calculated using the trapezoidal method. Maximum concentrations (C_{max}) are the observed values.

Statistical Analysis Data are expressed as the mean± standard deviation (S.D.). Statistical comparisons were performed using analysis of variance with Scheffe's test. Linear regression analysis with Pearson's coefficients was performed to investigate correlations.

RESULTS

Subject Demographic Characteristics and Disposition Twenty-four subjects were enrolled in this study; the cohort included 13 males (54%), with a mean age of 24 (23–32) years. The mean body weight was 60 (40–92) kg, mean height was 1.69 (1.53–1.84) m, and mean BMI was 20.8 (16.3–28.7) kg/mm^2. No subjects were withdrawn from the study. No adverse effects are observed.

Pharmacokinetics Figure 1 shows representative HPLC chromatograms of plasma afterenzymatic hydrolysis. The main pharmacokinetic data of curucmin in healthy volunteers (n=24) administered a single oral dose of each curcumin bevarage are presented in Table 3, and mean plasma concentrations of curcumin for each drinkable preparation over time are shown in Fig. 2. For all treatments, plasma curcumin levels were quantifiable 30 min after administration. Peak plasma concentrations for all drinkable preparations were not detected during 8 h. At all points, plasma levels of curcumin were higher in A than B, C, and D. This difference was apparent in males. In females, plasma levels of cucumin were significantly higher in A than C and D, and tended to be higher in A than

Table 1. Composition of Each Drink Containing Curcumin

Sample	Curcumin content (display value)	Composition
Drink A	30 mg/100 mL	Water, sugar group (high-fructose corn syrup, sugar), cinnamon extract, ginger, alanine, acidulant, turmeric colorant (Theracurmin), vitamin C, flavor, niacinamide, sweetener (licorice, sucralose), calcium pantothenate, vitamin B_6, vitamin B_2, vitamin B_1, vitamin B_{12}
Drink B	30 mg/100 mL	Water, sugar, turmeric extract, Korean ginseng extract, alanine, trehalose, citric acid, arginine, flavor, turmeric colorant, vitamin C, sweetener (sucralose), niacin, vitamin B_2, vitamin B_6, vitamin P, phenylalanine, isoleucine, threonine, monosodium glutamate
Drink C	40 mg/120 mL	Water, high-fructose corn syrup, dextrin, *Curcuma longa* extract, *Curcuma zedoaria* extract, acidulant, vitamin C, polysaccharide thickner, turmeric colorant, inositol, flavor, cyclic oligosaccharide, sweetener (sucralose, acesulfame potassium, thaumatin), niacin, vitamin B_1, vitamin E, emulsifier, vitamin B_6
Drink D	30 mg/100 mL	Water, high-fructose corn syrup, dextrin, *Curcuma longa* extract, salt, acidulant, vitamin C, polysaccharide thickner, inositol, turmeric colorant, flavor, cyclic oligosaccharide, niacin, sweetener (sucralose, acesulfame potassium, thaumatin), vitamin E, vitamin B_6, antioxidant (catechin)

Table 2. Assignment of Volunteers in Cross-over Study

	Group 1 (n=6)	Group 2 (n=6)	Group 3 (n=6)	Group 4 (n=6)
1st week	Drink A	Drink B	Drink C	Drink D
2nd week	Drink B	Drink C	Drink D	Drink A
3rd week	Drink C	Drink D	Drink A	Drink B
4th week	Drink D	Drink A	Drink B	Drink C

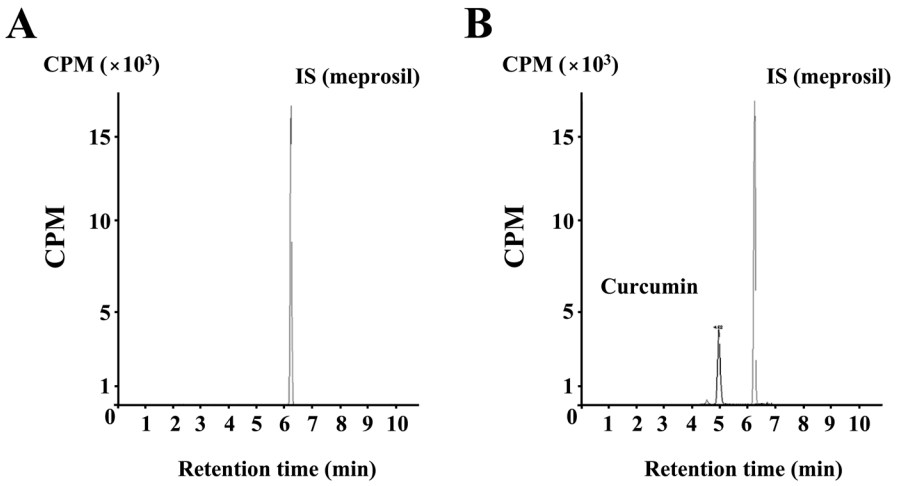

Fig. 1. Representative Examples of HPLC Chromatograms

Curcumin was identified in plasma after enzymatic hydrolysis. 0 h (A) and 1 h (B) after oral intake of Theracurmin beverage, IS: internal standard.

Table 3. Pharmacokinetic Parameters of Curcumin in the Plasma

	C_{max} (ng/mL)	$AUC_{0-0.5h}$ (ng/mL)	AUC_{0-1h} (ng/mL)	AUC_{0-2h} (ng/mL)	AUC_{0-4h} (ng/mL)	AUC_{0-8h} (ng/mL)
Drink A	25.5±12.2*,#,†	2.0±1.7	6.8±4.9#,†	18.3±11.5#,†	46.0±27.8*,#,†	121.2±65.6*,#,†
Male	28.3±13.8*,#,†	2.0±1.1	6.8±3.9	18.4±10.8#,†	47.3±28.7#,†	124.6±72.2*,#,†
Female	22.3±9.8#,†	2.1±2.3	6.7±6.3	17.3±13.3#,†	43.3±27.8#,†	110.6±61.9#,†
Drink B	14.9±5.4#,†	1.4±0.7	4.9±2.4	14.0±6.3#,†	33.9±14.2#,†	79.5±31.4#,†
Male	14.5±5.3	1.4±0.8	5.1±2.7	13.3±6.8	29.6±12.9	68.9±31.0
Female	15.4±5.8†	1.4±0.7	4.9±2.1	14.8±6.3	36.9±15.7†	83.8±34.2†
Drink C	8.6±4.9	1.2±0.6	3.5±1.5	7.7±3.9	16.8±10.6	40.9±24.9
Male	8.8±5.2	1.2±0.4	3.5±1.5	7.8±4.1	15.3±10.2	38.0±24.5
Female	8.4±4.7	1.2±0.7	3.4±1.7	7.7±3.9	18.7±11.4	44.3±27.3
Drink D	6.7±3.0	1.3±0.5	3.7±1.3	7.5±2.8	13.9±6.6	30.1±14.4
Male	7.2±3.7	1.3±0.5	3.5±1.2	6.9±2.6	12.6±6.6	30.4±17.6
Female	6.2±2.0	1.4±0.6	3.9±1.4	8.2±3.0	15.4±6.6	29.8±11.3

The data are shown as the mean value±standard deviation. *AUC*: Area under the curve; C_{max}: Maximal concentration. *$p<0.05$ *versus* drink B. #$p<0.05$ *versus* drink C. †$p<0.05$ *versus* drink D.

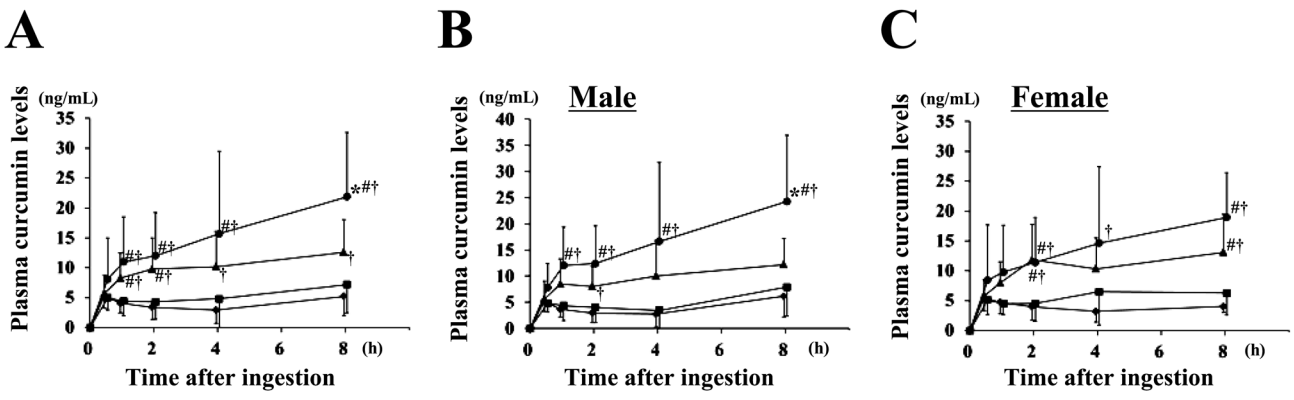

Fig. 2. Change in the Plasma Concentration of Curcumin in Healthy Volunteers

●, drink A. ▲, drink B. ■, drink C. ◆, drink D. Each point and bar represents the mean±S.D. (n=24). *$p<0.05$ *versus* drink B. #$p<0.05$ *versus* drink C. †$p<0.05$ *versus* drink D.

B. As shown in Table 3, the AUC_{0-8h} values of A became about 1.5 to 4.0-fold higher than those of the other 3 kinds of curcumin beverage. Plasma C_{max} (0–8 h) of Theracurmin were 1.8 to 3.8 times higher than those of the other 3 curcumin beverages (Table 3). No significant differences were observed between genders. The results of this study indicate that the bioavailability as measured by the *AUC* is significantly higher with Theracurmin beverage than the other 3 curcumin beverages.

To compare the absorption rates, we evaluated AUC_{0-2h}, AUC_{0-4h}, and AUC_{0-8h}. The AUC_{0-2h} of A was 1.3-, 2.4-, and 2.4-fold higher than that of B, C, and D, respectively, the AUC_{0-4h} was 1.4-, 2.7-, and 3.3-fold higher, and the AUC_{0-8h} was 1.5-, 3.0-, and 4.0-fold higher in A than B, C, and D. The

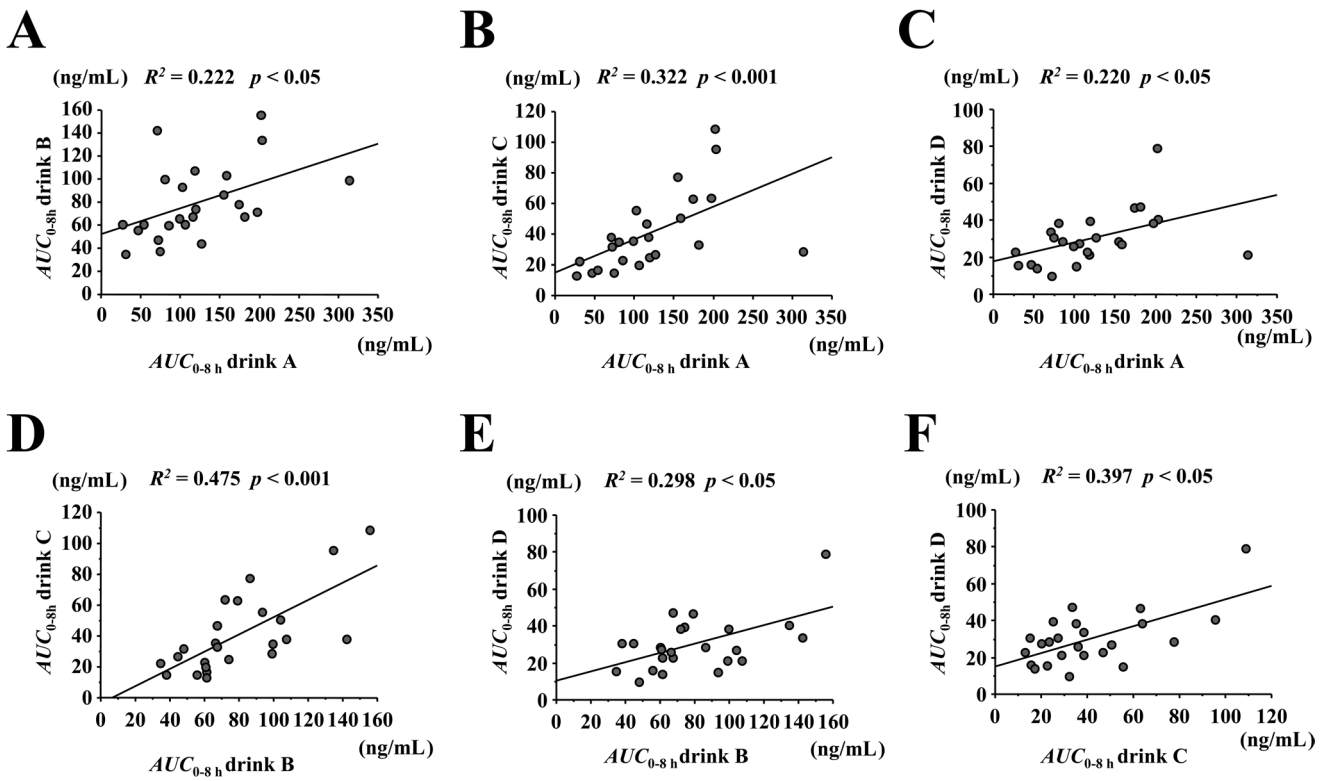

Fig. 3. Correlations of $AUC_{0-8\,h}$ Values of Curcumin among A, B, C, and D

differences became larger as the duration was longer.

The differences of $AUC_{0-8\,h}$ among volunteers were marked. To determine whether low or high absorption efficiency in each individual is shared by all 4 types of curcumin beverage, we examined $AUC_{0-8\,h}$ correlations among these beverages in each individual. As shown in Fig. 3, significant good correlations were observed between any 2 types of curcumin beverage. These findings suggest that individual differences in the pharmacokinetics of curcumin are shared by all 4 types of curcumin beverage.

DISCUSSION

We have newly created a drinkable preparation of Theracurmin, a highly absorptive curcumin. The present double-blind, 4-way crossover study in healthy volunteers demonstrated that the Theracurmin beverage yielded higher C_{max} and *AUC* than the other curcumin beverages sold in Japan. This indicates that Theracurmin beverage possesses a higher absorption efficiency compared with other curcumin beverages.

The problem of curcumin after oral administration is its poor systemic bioavailability, due to its low solubility in water as many other natural polyphenols, and its rapid metabolism. Recently, drug delivery systems accompanied by nanoparticle technology have emerged as prominent solutions to the bioavailability as therapeutic agents. Although nanoparticle-based delivery systems might be suitable for highly hydrophobic agents like curcumin to circumvent the pitfalls of poor aqueous solubility, very few studies have been reported regarding curcumin nanoparticles. A polymer-based nanoparticle of curcumin, "nanocurcumin," with a particle size of less than 100 nm size was synthesized. While nanocurcumin works well as curcumin *in vitro*, no efficacy of nanaocurcumin over free curcumin *in vivo* has been reported.[24] Curcuminoid-loaded solid lipid nanoparticles were developed for long-term stability at room temperature and reduced light and oxygen sensitivity.[25] To increase the bioavailability of curcumin, nanoparticles encapsulating curcumin have been prepared by the emulsion technique.[26] These include an optimized polylactic-*co*-glycolic acid nano-formulation,[27] dextran sulfate-chitosan nanoparticles with curcumin,[28] polymeric nanoparticle-encapsulated curcumin,[29,30] and water-dispersible hybrid nanogels.[31] However, these nanoparticle-based systems for curcumin delivery are still in their infancy, and much progress is warranted in this area. Here, we have generated highly absorptive curcumin, Theracurumin, using a micro-particle and surface-controlled colloidal dispersion method, which markedly improves oral bioavailability. The *AUC* after the oral administration of Theracurmin was more than 40- and 27-fold higher than that of curcumin powder in rats and humans, respectively.[22] Thus, Theracurmin may be useful to exert clinical benefits in humans at lower dosages.

We reported that the plasma curcumin level reaches a maximum at 1 h after the oral administration of 30 mg of Theracurmin in healthy volunteers.[22] Many reports indicate that oral administration of curcumin in humans and rodents results in peak plasma levels at around 1 h after the intake.[32–35] However, in this study, drinkable types of curcumin including the Theracurmin beverage yielded maximum plasma curcumin levels more than 8 h after drinking. In all experiments of pharmacokinetics, subjects were in completely fasting states before and after taking samples. Thus, the influence of meals could be discounted. A number of clinical studies demonstrated that concomitant food and drug intake reduces C_{max} but increases *AUC*. These findings suggest that food intake increases the overall bioavailability but reduces the peak systemic exposure

relative to fasting conditions.[36,37] Therefore, one of the reasons for the delayed peak is that drinkable types of curcumin contain many components other than curcumin, which may affect its absorption speed.[21] In normal life, it is expected that curcumin beverages are taken before and after meals, and their absorption speeds should be slower than those indicated by pharmacokinetic experiments. Another reason for this delay in the curcumin peak is enterohepatic circuration. When curcumin was given orally to rats, most of it was excreted in the feces and negligible amounts were found in the urine.[38] Intravenous and intraperitoneal administrations of curcumin resulted in biliary excretion in rats.[39] These data indicate that curcumin is re-absorbed from feces and that the plasma concentration of curcumin is maintained at high levels for a long time. This may be useful to exert and sustain the physiological effects of the drinkable types of curcumin, especially Theracurmin beverage.

In this study, we compared plasma levels of curcumin in Theracurmin beverage and those of other curcumin beverages sold in Japan. We reported that the plasma level of curcumin is significantly higher after the oral administration of Theracurmin than that of curcumin powder in humans.[22] Since the compositions differ between Theracurmin beverage and other curcumin beverages (Table 1), such differences may affect curcumin levels. However, as the precise composition, origin, extraction method, and curcumin modification of each beverage are unclear, the substitution of curcumin beverages with Theracurmin was impossible. Further pharmacokinetic studies are needed to clarify possible effects of the beverage components on blood curcumin levels.

Curcumin is the active ingredient of the dietary spice turmeric and has been consumed for medicinal purposes for thousands of years.[40,41] Modern science has shown that curcumin modulates various signaling molecules, including inflammatory molecules, transcription factors, enzymes, protein kinases, and protein reductases. Moreover, curcumin has been reported to possess bioactivity, such as anti-inflammatory, anti-oxidant, pro-apoptotic, chemopreventive, chemotherapeutic, anti-proliferative, wound healing, anti-nociceptive, antiparasitic, and anti-malarial properties. Animal studies have suggested that curcumin may be active against a wide range of human diseases, including diabetes, obesity, neurologic and psychiatric disorders, and cancer, as well as chronic illnesses affecting the eyes, lungs, liver, kidneys, and gastrointestinal and cardiovascular systems. Curcumin has been shown to have the potential to improve many diseases in human research as well as experimental studies including cultured cells and animal models. In addition, more than 90 clinical trails using supplemental curcumin are on-going in many countries. Furthermore, curcumin is used as a supplement in several countries, including India, Japan, the United States, Thailand, China, Korea, Turkey, South Africa, Nepal, and Pakistan. However, this inexpensive, apparently well-tolerated, and potentially active curcumin has not yet been approved for the treatment of any human disease. Many clinical trials evaluating curcumin's safety and efficacy against human ailments have already been completed.[42] In the near future, curcumin may be used not only in health foods, but also medical agents.

We have shown that curcumin exhibits biological activity to prevent the deterioration of systolic functions in rat heart failure models at C_{max} with 10.7±1.7 and 5.0±2.4 ng/mL.[23] In this study, C_{max} of Theracurmin beverage was 25.5±12.2 ng/mL, which is higher than those with protective effects on the heart. We previously examined whether Theracurmin exerts effects on alcohol metabolism after drinking ethanol in healthy volunteers. We demonstrated that Theracurmin could reduce plasma levels of acetaldehyde, a product of ethanol.[22] These data indicate that Theracurmin directly affects the metabolism of acetaldehyde and accelerates the detoxification of ethanol. The AUC_{0-6h} value which reduces the plasma concentration of acetaldehyde after ethanol consumption in human is 113±61 ng/mL·h and almost comparable to AUC_{0-8h} (121.2±65.6 ng/mL·h) after taking Theracurmin beverage in this study.[22] Therefore, Theracurmin beverage may be useful to obtain plasma curcumin levels sufficient to exert beneficial effects such as alcohol detoxication. Moreover, many studies indicate that curcumin shows powerful hepatoprotective effects against oxidative damage caused by several hepatotoxins including ethanol.[43] In those studies, curcumin, not only attenuated lipid peroxidation but also recovered the activity of endogenous antioxidative defense system.[44–49] Therefore, Theracurmin might provide protection against alcoholic liver damage.

These findings demonstrate that Theracurmin beverage shows the highest bioavailability among currently available preparations of curcumin. Thus, it may be useful to exert its physiological benefits in humans at lower dosages.

Acknowledgment This work was supported in part by a Grant-in-Aid to K. Hasegawa, T. Morimoto, Y. Sunagawa, and Y. Katanasaka for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and from the Takeda Science Foundation, Annual Research Award Grant of Japanese Society of Anti-Aging Medicine, and joint research funding from the Theravalues Corporation.

Conflict of Interest Contributing authors Y. Otsuka, T. Hamada and A. Imaizumi are full-time employees of Theravalues Corporation. Y. Nonaka, T. Fuwa and T. Teramoto are full-time employees of Suntory Beverage and Food Limited. The other authors declare that no competing interests exist.

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