Innovative Preparation of Curcumin for Improved Oral Bioavailability

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Curcumin is a polyphenol that is commonly used for its perceived health benefits. However, the absorption efficacy of curcumin is too low to exhibit beneficial effects. We have successfully developed a highly absorptive curcumin dispersed with colloidal nano-particles, and named it THERACURMIN. The absorption efficacy of THERACURMIN was investigated and compared with that of curcumin powder. The area under the blood concentration–time curve (*AUC*) after the oral administration of THERACURMIN was found to be more than 40-fold higher than that of curcumin powder in rats. Then, healthy human volunteers were administered orally 30 mg of THERACURMIN or curcumin powder. The *AUC* of THERACURMIN was 27-fold higher than that of curcumin powder. In addition, THERACURMIN exhibited an inhibitory action against alcohol intoxication after drinking in humans, as evidenced by the reduced acetaldehyde concentration of the blood. These findings demonstrate that THERACURMIN shows a much higher bioavailability than currently available preparations. Thus, THERACURMIN may be useful to exert clinical benefits in humans at a lower dosage.

Key words   curcumin; bioavailability; nano-particle colloidal dispersion; absorption efficiency

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is the active ingredient of turmeric, which has a long history of being consumed as a dietary spice.[1] In addition, turmeric is widely used in traditional Indian medicine to treat biliary disorders, anorexia, cough, diabetic complications, hepatic disorders, rheumatism, and sinusitis.[2] Extensive investigation over the past five decades has indicated that curcumin reduces blood cholesterol, prevents low-density lipoprotein oxidation, inhibits platelet aggregation, suppresses thrombosis and myocardial infarction, suppresses symptoms associated with type 2 diabetes, rheumatoid arthritis, multiple sclerosis, and Alzheimer's disease, inhibits human immunodeficiency virus (HIV) replication, enhances wound healing, protects against liver injury, increases bile secretion, protects from cataract formation, and protects against pulmonary toxicity and fibrosis.[3—5] Evidence indicates that the divergent effects of curcumin are dependent on its pleiotropic molecular effects.

In spite of these attractive properties of curcumin, information on the therapeutic efficiency of curcumin has been limited, partly due to its poor oral bioavailability.[6] Curcumin was found to be poorly soluble in water, the maximum solubility of which in aqueous buffer (pH 5.0) was reported to be as low as 11 ng/ml.[7] The limited solubility of curcumin, as well as extensive systemic metabolism, could be responsible for the low bioavailability of curcumin after oral delivery.[8—10] In addition, curcumin in solution may be sensitive to UV light, and so marked photochemical degradation could occur under UV exposure,[11] leading to difficulty in its handling for clinical use.

A number of efforts have been made to design a soluble formulation of curcumin, but no suitable delivery options have been found so far. We have developed an effective preparation of curcumin, a nano-particle colloidal dispersion, with improved oral bioavailability, and named it THERACURMIN. It has the following unique properties: 1) it is an effective preparation for new health care products (beverages, food, and supplements) which may be taken at a much lower dosage; 2) it is soluble in water, which is a must for an effective beverage product; 3) the preparation is highly stable in light (UV), and, therefore, can be put into transparent PET bottles; 4) it is heat-stable, including high temperature sterilization conditions; 5) the preparation has no unpleasant odor or taste.

The main purpose of this study was to provide evidence to support the improved bioavailability and alcohol-toxicity-reducing effect of THERACURMIN through oral delivery. We evaluated the plasma pharmacokinetics of this new curcumin preparation and compared the results with curcumin powder after oral administration in rats and healthy human subjects. We also investigated the effect of THERACURMIN on the toxicity of alcohol following drinking.

MATERIALS AND METHODS

Preparation of Curcumin Powder and THERACURMIN   Curcumin powder was extracted from Indian turmeric by using alcohol. THERACURMIN was prepared as follows; first, gum ghatti, mainly consists of polysaccharides, obtained from the exudation of ghatti trees, was dissolved in water to make gum ghatti solution. Curcumin powder was mixed into this solution, and water and glycerin was added to adjust the weight. This mixture was ground by a wet grinding mill (DYNO-MILL® KDL, Willy A Bachofen AG), and then,

dispersed by a high-pressure homogenizer (Homogenizer 15MR-8TA, APV Gaulin). After this procedure, stable THERACURMIN was obtained. THERACURMIN consisted of 10 w/w% of curcumin, 2% of other curcuminoids such as demethoxycurcumin and isdemethoxycurcumin, 46% of glycerin, 4% of gum ghatti, and 38% of water.

Chemicals Curcumin powder, mepronil, β-glucuronidase (from *Helix pomatia*), distilled water (H_2O), acetonitorile (MeCN), methanol (MeOH), formic acid (FA), sodium acetate, and chloroform were purchased from Wako (Osaka, Japan).

Measurement of Particle Size The particle size of curcumin was measured by employing a laser diffraction scattering method using Microtrac MT-3000II (Microtrac Inc., Montgomeryville, U.S.A.).

Experimental Design for Oral Administration in Rats Male Sprague-Dawley rats weighing 250—290 g were randomly assigned to four groups, each containing three rats. Curcumin powder was directly suspended in the 1% gum ghati solution. The THERACURMIN solution containing 10% of curcumin was dispersed in the 1% gum ghati solution. The final content of curcumin in the gum ghati solution was 1% in both curcumin powder and THERACURMIN. The first and second groups were given curcumin powder at a dosage of 50 and 300 mg curcumin/kg body weight, respectively. The third and fourth groups were given THERACURMIN at 50 and 300 mg/kg body weight (containing 5 and 30 mg/kg body weight of curcumin, respectively), respectively. The samples were orally administered to rats by direct stomach intubation using gastric catheters. Directly before oral administration, and 1, 2, 4, 6, and 24 h after administration, blood was taken from the tail of rats and placed into heparinized tubes. Plasma was immediately prepared by centrifugation at 1000 *g* for 10 min at 4 °C and stored at −20 °C until use.

Oral Administration in the Clinical Trial Thirty milligrams of curcumin powder was wrapped by small thin starch sheet (Oblate®) into a small size and orally given with 100 ml of water. The THERACURMIN solution containing 30 mg of curcumin was dispersed in 100 ml of water and orally administrated.

Clinical Trial Design for Oral Administration in Human Volunteers Male and female volunteers, aged between 30 and 59 years, with a body mass index ranging from 18—30 were selected. The selected subjects were 8 males and 6 females (ages=44.1±8.5 years, body mass index=23.7±3.0 kg/m²). Subjects were not taking any medications before or during this study. Both curcumin powder and THERACURMIN in liquid form were administered orally at a dose of 30 mg of curcumin. Subjects were randomly assigned to dose groups, with 7 subjects in each treatment group. Blood samples (0.5 ml) were collected in heparinized tubes before dosing and at 1, 2, 4, 6, and 24 h after curcumin was administered. The plasma was separated from blood cells immediately after blood collection and kept at −20 °C until use. We did not include a treatment control in this study. The study protocol and comprehensive written informed consent used in this study protocol were approved by the Ethics Committee of Akihabara Medical Clinic (Tokyo, Japan) prior to the start of the study.

Clinical Trial Design for the Effects of THERACUR-MIN on Alcohol Metabolism in Healthy Human Volunteers Seven subjects (7 males, ages=42.7±5.2 years, body mass index=22.7±3.9 kg/m²) were divided into 2 groups consisting of 4 and 3. Group 1 (*n*=4) subjects were administered 100 ml of mineral water containing THERACURMIN 30 mg, and group 2 (*n*=3) subjects were administered mineral water only following the ingestion of 0.5 ml/kg of ethanol. The blood-ethanol level and blood-acetaldehyde level associated with ethanol metabolism were measured before and 30, 60, 120, and 180 min after ethanol consumption. After a wash-out period of 1 week, the subjects were crossed-over, and group 1 subjects received mineral water and group 2 subjects received 100 ml of mineral water containing 30 mg of THERACURMIN. We did not include curcumin powder in this study. The protocol and comprehensive written informed consent were approved by the Institutional Review Board of Chiyoda Paramedical Clinic (Tokyo, Japan) prior to the start of the study.

Plasma Concentration of Curcumin The HPLC-MS/MS system consisted of 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). Samples were subjected to a C-18 column-Atlantis T3 (2.1×150 mm, 3 μm) (Waters, Milford, U.S.A.) using a gradient of binding solvent (0.05% FA/H_2O) and elution solvent (0.05% FA/MeCN) at a flow rate of 0.2 ml/min and a column temperature of 40 °C. The separation of samples was conducted employing a 35-min linear gradient (5—95% elution solvent). The mass spectrometer was operated under MRM mode with collision energy of 23 eV for curcumin and 33 eV for mepronil. The transitions (precursor to product) monitored were m/z 369→285 for curcumin, and 270→119 for mepronil. Chromatograms were integrated using ANALYST version 1.5 software.

Stock solutions of curcumin and mepronil (IS, internal standard) were prepared separately both at a concentration of 1000 ng/ml in MeOH. The stock solution of mepronil was further diluted with 55% MeOH to prepare a calibration standard at a concentration of 100 ng/ml. The stock solution of curcumin was further diluted with 62% MeOH to prepare a calibration standard at a concentration of 200 ng/ml. Curcumin solution (200 ng/ml) was diluted with 50% MeOH to prepare the following standard solutions: 0.4, 0.8, 1.6, 3.1, 6.3, 12.5, 25.0, 50.0, and 100.0 ng/ml. These solutions were mixed with IS solution (100 ng/ml) at a ratio of 1:1 to prepare IS-containing calibration samples of 0.2—100.0 ng/ml (curcumin) and 50 ng/ml (mepronil). The same stock solution (1000 ng/ml) of mepronil was further diluted with MeOH to prepare the IS working solution at a concentration of 500 ng/ml.

Sample Preparation A 0.1 ml aliquot of each plasma sample collected from rats and human subjects was transferred to a 10 ml glass tube and then 0.11 ml of 0.1 M sodium acetate buffer (pH 5.0) containing 1000 U β-glucuronidase was added. The resulting solutions were incubated to hydrolyze the curcumin conjugates at 37 °C for 1 h. After 10 μl of IS working solution (500 ng/ml) was added, a 0.5 ml volume of chloroform as an extraction solvent was added. The sample was vortexed for 1 min, followed by ultrasonic vibrations for 15 min and then centrifugation at 1610×*g* for
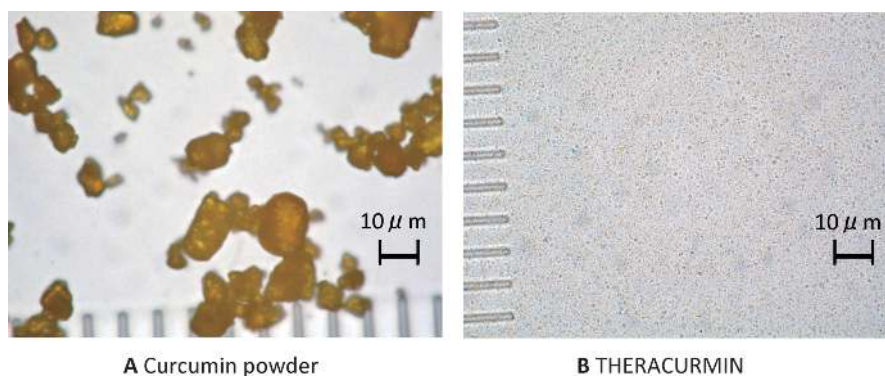
Fig. 1. Microscopic Images of Curcumin Powder (A) and THERACURMIN (B)

Both were dispersed in water and observed under the optical microscope at 1 h after the dispersion (1000× magnification).

5 min. The organic layer was transferred to a 1 ml glass tube and evaporated to dryness using a centrifuge concentrator. The dried extract was reconstituted in 100 μl of 50% MeCN containing 0.05% FA and then centrifuged at 7700 g for 10 min. A 10 μl aliquot of supernatant of reconstituted sample solution was injected into a chromatographic system.

Concentration of Ethanol and Acetaldehyde in Blood The ethanol and acetaldehyde content of blood or breath ethanol was determined by headspace-gas chromatography with isopropyl alcohol as an internal standard.

Statistical Analysis Data are expressed as the mean$\pm$ standard deviation (S.D.). The significance of differences was analyzed using the t-test. A value of $p < 0.05$ was considered significant.

RESULTS

Mean Particle Size and Size Distribution of THERA-CURMIN and Curcumin Powder The mean particle size of THERACURMIN (D50% diameter) was 0.19 μm. The mean particle size of curcumin powder was 22.75 μm.

The Microscopic Image and Dispersion Stability of THERACURMIN Curcumin powder or THERACURMIN was dispersed in water. Figure 1 indicates microscopic images of curcumin powder (A) and THERACURMIN (B) at 1 h after the dispersion. Homogenized very small particles were seen in THERACURMIN, whereas curcumin powder showed crystal aggregates with various sizes around several dozen micrometers. With regard to dispersion stability (Fig. 2), THERACURMIN was stably dispersed even at 28 d after the dispersion, but curcumin powder began to precipitate at 1 h after the dispersion, and the precipitations were clearly seen at 1 d.

Plasma Concentration of Curcumin in Rats Figure 3 shows the plasma concentration–time profiles of curcumin in rats after oral administrations of THERACURMIN and curcumin powder, and the pharmacokinetic parameters including C_{max} (maximum concentration), T_{max} (time to reach the maximum concentration), and AUC_{0-6h} (area under the blood concentration *versus* time curve) are indicated in Table 1. As shown in Fig. 3, plasma curcumin concentrations were significantly higher in rats administered THERACURMIN than curcumin powder, at each dose. The AUC_{0-6h} values at THERACURMIN doses of 50 and 300 mg/kg were 2248$\pm$ 380 and 5734$\pm$1697 ng·h/ml, respectively, while the AUC
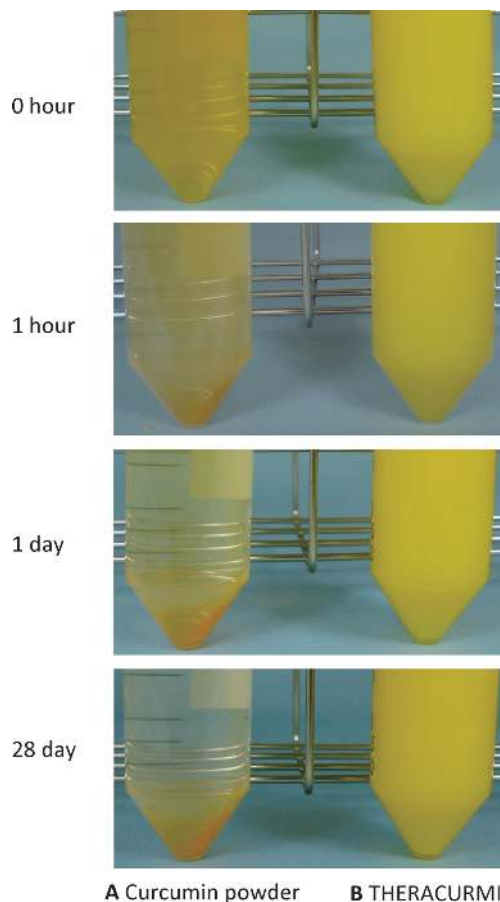

Fig. 2. Dispersion Stability of Curcumin Powder and THERACURMIN

Thirty milligrams of curcumin powder or 300 mg of THERACURMIN (containing 30 mg of curcumin) were dispersed in 100 ml of water, and observed at 0 h (just after the dispersion), 1 h, 1 d, and 28 d after the dispersion.

values at curcumin powder doses of 50 and 300 mg/kg were 51.1$\pm$25 and 134$\pm$114 ng/ml·h, respectively. Thus, it was confirmed that THERACURMIN raised the plasma concentration of curcumin by 39.8—81.7 times at 1—2 h after administration compared with curcumin powder. The AUC_{0-6h} values of THERACURMIN became about 42.8—44 times higher than those of curcumin powder. These results suggest that THERACURMIN markedly increased the absorption in rats, and the bioavailability of THERACURMIN was more than 40-fold higher than that of curcumin powder, as meas-

ured by the *AUC*.

Plasma Concentration of Curcumin in Human Subjects Figure 4 shows the plasma concentration–time profiles of curcumin in human volunteers after the oral administration of THERACURMIN and curcumin powder. Pharmacokinetic parameters including C_{max}, T_{max}, and AUC_{0-6h} are shown in Table 2. No adverse effect was observed in this study. The plasma concentrations of curcumin were signifi-

cantly higher at the THERACURMIN 30 mg dose level than at the curcumin powder 30-mg dose level. The plasma concentrations of curcumin at C_{max} at the THERACURMIN 30 mg dose level were 29.52 ± 12.86 ng/ml. The curcumin powder 30-mg dose level achived 1.84 ± 2.03 ng/ml. The AUC_{0-6h} values at each dose level were 113.04 ± 61.33 and 4.14 ± 3.88 ng/ml·h, respectively. The AUC_{0-6h} values at the THERACURMIN 30-mg dose level was 27.3 fold higher than at the curcumin powder 30-mg dose level. This sug-
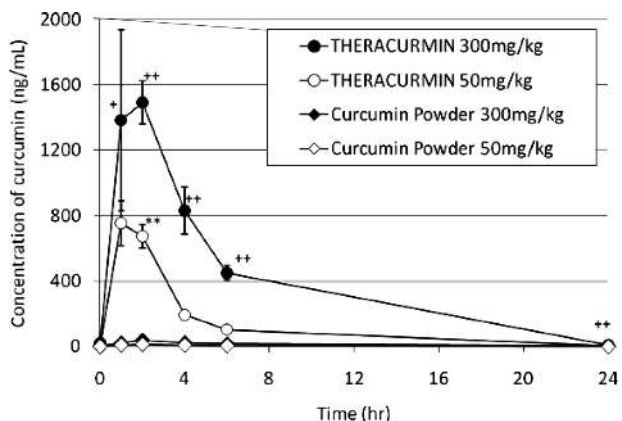

Fig. 3. Concentration of Curcumin in Rat Plasma after the Oral Administration of THERACURMIN and Curcumin Powder

●, THERACURMIN 300 mg/kg. ○, THERACURMIN 50 mg/kg. ◆, Curcumin powder 300 mg/kg. ◇, Curcumin powder 50 mg/kg. Data represent mean±S.E. (*n*=3). ∗∗*p*<0.01 (THERACURMIN 50 mg/kg *vs.* curcumin powder 50 mg/kg). †*p*<0.05 (THERACURMIN 300 mg/kg *vs.* curcumin powder 300 mg/kg). ‡*p*<0.01 (THERACURMIN 300 mg/kg *vs.* curcumin powder 300 mg/kg).
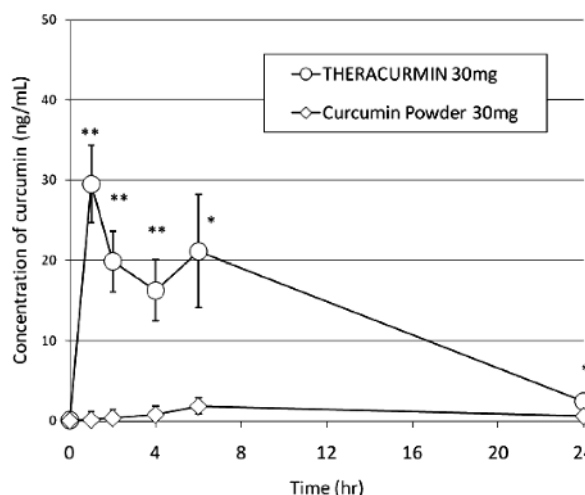

Fig. 4. Concentration of Curcumin in Human Plasma after the Oral Administration of THERACURMIN and Curcumin Powder

○, THERACURMIN 30 mg. ◇, Curcumin powder 30 mg. Data represent mean±S.E. (*n*=7). ∗*p*<0.05 (THERACURMIN 30 mg *vs.* curcumin powder 30 mg). ∗∗*p*<0.01 (THERACURMIN 30 mg *vs.* curcumin powder 30 mg).

Table 1. Pharmakokinetic Parameters of THERACURMIN Following Oral Administration in Rat

	n	Dose (mg/kg)	AUC_{0-6h} (ng/ml·h) mean±S.D.	C_{max} (ng/ml) mean±S.D.	T_{max} (h)
Curcumin powder	3	50	51.1±25	13.0±5.8	2
	3	300	134±114	37.4±36.1	2
THERACURMIN	3	50	2248±380	764±231	1
	3	300	5734±1697	1697±578	2

Table 2. Pharmacokinetic Parameters of THERACURMIN Following Oral Administration in Healthy Human

	n	Dose (mg/kg)	AUC_{0-6h} (ng/ml·h) mean±S.D.	C_{max} (ng/ml) mean±S.D.	T_{max} (h)
Curcumin powder	7	30	4.1±7	1.8±2.8	6
THERACURMIN	7	30	113±61	29.5±12.9	1

A

B
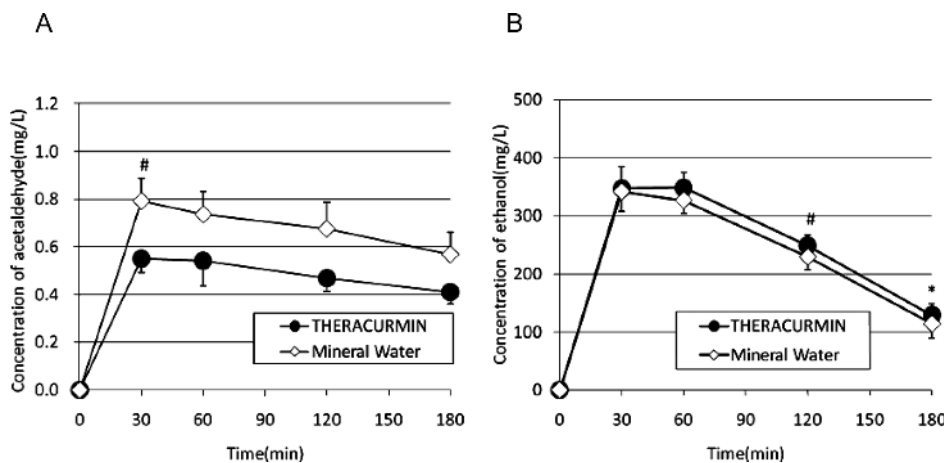

Fig. 5. (A) Concentration of Acetaldehyde in Human Plasma after Ethanol Consumption

●, THERACURMIN 30 mg. ◇, Mineral water. Data represent mean±S.E. (*n*=7). #*p*<0.01 (THERACURMIN 30 mg *vs.* mineral water).

(B) Concentration of Ethanol in Human Plasma after Ethanol Consumption

●, THERACURMIN 30 mg. ◇, Mineral water. Data represent mean±S.E. (*n*=7). #*p*<0.01 (THERACURMIN 30 mg *vs.* mineral water). ∗*p*<0.05 (THERACURMIN 30 mg *vs.* mineral water).

gested that the absorption of THERACURMIN was dose-dependent. The results of this study indicate that the bioavailability as measured by the *AUC* of THERACURMIN was at least 27-fold higher compared to curcumin powder in both rats and humans.

Effects of THERCURMIN on Alcohol Metabolism in Healthy Human Subjects   We investigated the effect of THERACURMIN after alcohol drinking. Figures 5A and B show the concentration of acetaldehyde and ethanol in human volunteers after ethanol consumption. The plasma concentration of acetaldehyde was significantly lower in subjects who drank curcumin compared with mineral water. In the case of ethanol, both groups showed similar results. There was no significant effect of curcumin on ethanol reduction. Therefore, the THERACURMIN preparation may directly act on acetaldehyde reduction following alcohol intake.

DISCUSSION

The potential efficacy of curcumin to treat a variety of diseases is an interesting subject of research today. A number of clinical trials have determined the potential of curcumin to treat numerous disorders. Researchers have claimed that the enhancement of curcumin bioavailability can bring this natural molecule to the forefront of therapeutic agents for the treatment of human disorders.

Curcumin exhibits pharmacological safety and efficacy; therefore, curcumin is expected to be a potential compound for the treatment and prevention of a wide variety of diseases. In spite of its efficacy and safety, curcumin has not yet been approved as a therapeutic agent, its low-level bioavailability has been highlighted as a major problem. The fact that curcumin exhibits poor bioavailability has been well documented by Anand *et al.*[1] The major reasons for the low bioavailability of curcumin are its poor water solubility and absorption, rapid metabolism, and rapid systemic elimination.

A study by Yang *et al.*[6] showed that 10 mg/kg of curcumin given intravenously to rats yielded a maximum serum curcumin level of $0.36\pm0.05\,\mu$g/ml, whereas 500 mg/kg of curcumin administered orally only yielded a $0.06\pm0.01\,\mu$g/ml maximum serum level in rats. The oral bioavailability of curcumin was about 1%. Therefore, several delivery strategies, including adjuvants, nanoparticles, liposomes, micelles, and phospholipid complexes, are currently being evaluated to enhance the bioavailability and biological activity of curcumin.[12—16] Sharma *et al.*[10] showed that there was no detectable curcumin or its metabolites in the blood or urine after the administration of 440—2200 mg of curcuma extract per day (containing 36—180 mg of curcumin) for up to 29 d to patients with advanced colorectal cancer. Cheng *et al.*[17] demonstrated that the peak concentrations of curcumin in the serum after administration of 4, 6, and 8 g of curcumin (given in the form of tablets obtained from a commercial source, with each tablet containing 500 mg curcumin) were 0.51, 0.64, and 1.77 mM, respectively. Moreover, these investigators found that doses below 4 g were barely detectable. Lao *et al.*[18] could not detect curcumin in the serum of volunteers given 0.5, 1.0, 2.0, 4.0, 6.0, or 8.0 g of curcumin. This was provided in a capsule form as a standardized powder

extract, obtained commercially, containing a minimum 95% concentration of the 3 curcuminoids of curcumin, bis-demethoxycurcumin, and demethoxycurcumin. However, these authors found that curcumin levels reached 50.5 and 51.2 ng/ml sera by 4 h in 2 subjects administered 10 and 12 g of curcumin, respectively.

In this study, we successfully formulated an innovative preparation of curcumin, THERACURMIN, and demonstrated that its oral bioavailability is at least nearly 30-times higher than that of curcumin powder in both rats and humans. Oral THERACURMIN yielded higher $C_{\max}$ and shorter $T_{\max}$ values, as well as a higher *AUC*. These results indicate that THERACURMIN enhanced gastrointestinal absorption as a result of colloidal dispersion. We used gum ghati, which rave rise to a water soluble and stable preparation of curcumin. To the best of our knowledge, this is the first report that curcumin exhibits such a high oral bioavailability (at least 27-times higher).

The consumption of alcoholic beverages has increased significantly during recent years. It has been estimated that almost 109 million Americans of 14 years of age or older drink alcoholic beverages on a regular basis, and about 18 million Americans may be practicing unsafe drinking and/or suffering from alcohol-related diseases.[19] Chronic alcohol drinking is associated with a number of diseases, including addiction-related neurobehavioral disorders, inflammatory dysregulation, an increase in susceptibility to infection,[20] and a number of liver, pancreas, and cardiovascular diseases.[21] The alcohol-induced inflammatory dysregulation may be casually related to the alcohol-weakened immune system and liver, pancreas, and cardiovascular diseases. Since an effective therapy for alcohol-related diseases is not yet available, the management of these diseases poses a serious health problem with staggering medical and socioeconomic consequences to society. It has been well-established that ethanol is readily absorbed from the gastrointestinal tract,[22] circulates rapidly, and is metabolized to acetaldehyde by enzymes in liver, such as alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH), respectively.[23,24]

After confirming the oral bioavailability of THERACURMIN, we extended the study to evaluate it effect on alcohol metabolism after drinking. This study was conducted involving 7 humans. We analyzed both ethanol and acetaldehyde concentrations, and found that only the acetaldehyde was significantly reduced due to THERACURMIN. Acetaldehyde, a by product of ethanol, causes discomfort, such as headache, nausea, *etc.* Therefore, its reduction must be correlated with the lowering of such discomfort. Hamano *et al.*[25] suggested that beverage supplementation with a turmeric extract would moderate the effect of alcohol consumption and reduce discomfort due to alcohol drinking, which supports our findings. Another study suggested that herbal mixtures containing curcumin partially suppressed alcohol-related inflammatory abnormalities.[26]

The results, obtained from both the rat and human studies, suggest that the new preparation, THERACURMIN, has a much higher absorption capacity (bioavailability) compared with curcumin powder. Therefore, this innovative curcumin preparation, especially at a lower dosage, compared with currently available products, may be an ideal candidate for an effective agent against inflammatory and/or other chronic dis-

eases. Hence, THERACURMIN may contribute to be development of effective neutraceuticals.

REFERENCES

1) Anand P., Kunnumakkara A. B., Newman R. A., Aggarmwal B. B., *Mol. Pharm.*, **4**, 807—818 (2007).
2) Chattopadhyay I., Biswas K., Bamdyopadhyay U., Banerjee R. K., *Curr. Sci.*, **87**, 44—53 (2004).
3) Aggarwal B. B., Kumar A., Bharti A. C., *Anticancer Res.*, **23**, 363—398 (2003).
4) Frunk J. L., Frye J. B., Oyarzo J. N., Kuscuoglu N., Wilson J., McCaffery G., Stafford G., Chen G., Lantz R. C., Jolad S. D., Solyom, A. M., Kiela, P. R., Timmermann B. N., *Arthritis Rheum.*, **54**, 3452—3464 (2006).
5) Morimoto T., Sunagawa Y., Kawamura T., Takaya T., Wada H., Nagasawa A., Komeda M., Fujita M., Shimatsu A., Kita T., Hasegawa K., *J. Clin. Invest.*, **118**, 868—878 (2008).
6) Yang K. Y., Lin L. C., Tseng T. Y., Wang S. C., Tsai T. H., *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **853**, 183—189 (2007).
7) Tonnesen H. H., Masson M., Loftsson T., *Int. J. Pharm.*, **244**, 127—135 (2002).
8) Perkins S., Verschoyle R. D., Hill K., Parveen I., Threadgill M. D., Sharma R. A., Williams M. L., Steward W. P., Gescher A. J., *Cancer Epidemiol. Biomarkers Prev.*, **11**, 535—540 (2002).
9) Sharma R. A., Euden S. A., Platton S. L., Cooke D. N., Shafayat A., Hewitt H. R., Marczlo T. H., Morgan B., Hemingway D., Plummer S. M., Gescher A. J., Steward W. P., *Clin. Cancer Res.*, **10**, 6847—6854 (2004).
10) Sharma R. A., McLelland H. R., Hill K. A., Ireson C. R., Euden S. A., Manson M. M., Pirmohamed M., Marnett L. J., Gescher A. J., Steward W. P., *Clin. Cancer Res.*, **7**, 1894—1900 (2001).
11) Tonnesen H. H., Karlsen J., van Henegouwen G. B., *Z. Lebensm Unters Forsch.*, **183**, 116—122 (1986).
12) Bisht S., Maitra A., *Curr. Drug Discov. Technol.*, **6**, 192—199 (2009).
13) Shaikh J., Ankola D. D., Beniwal V., Singh D., Ravi Kumar M. N. V., *Eur. J. Pharm. Sci.*, **37**, 223—230 (2009).
14) Takahashi M., Uechi S., Takara K., Askin Y., Wada, K., *J. Agric. Food Chem.*, **57**, 9141—9146 (2009).
15) Onoue S., Takanashi H., Kawabata Y., Seto Y., Hatanaka J., Timmermann B., Yamada S., *J. Pharm. Sci.*, **99**, 1871—81 (2009).
16) Antony B., Merina B., Iyer V. S., Judy N., Lennertz K., Joyal S., *Indian J. Pharm. Sci.*, **70**, 445—449 (2008).
17) Cheng A. L., Hsu C. H., Lin J. K., Hsu M. M., Ho Y. F., Shen T. S., Ko J. Y., Lin J. T., Lin B. R., Wu M. S., Yu H. S., Jee S. H., Chen G. S., Chen T. M., Chen C. A., Lai M. K., Pu Y. S., Pan M. H., Wang Y. J., Tsai C. C., Hsibh C. Y., *Anticancer Res.*, **21**, 2895—2900 (2001).
18) Lao C. D., RuffinIV M. T., Normolle D., Heath D. D., Murray S. I., Mailey J. M., Boggs M. E., Crowell J., Rock C. L., Brenner D. E., *BMC Complement. Altern. Med.*, **6**, 10 (2006).
19) "Mayo Clinic Report DS00340: Alcoholism," Mayo Clinic, Rochester, MN, 2004.
20) Frank J., Witte K., Schrodl W., Schutt C., *Alcohol Alcohol*, **39**, 386—392 (2004).
21) Bruno A., *Curr. Neurol. Neurosci. Rep.*, **3**, 40—45 (2003).
22) Godde W. H., Agrawal D. P., "Alcoholism of Medical and Genetics Aspects," Pergamon Press, 1989, pp. 3—57.
23) Higuchi S., Matsushita S., Murayama M., Takagi T., Hayashida M., *Am. J. Psychiatry*, **152**, 1291—1297 (1995).
24) Agrawal D. P., *Pathol. Biol.* (Paris), **49**, 703—709 (2001).
25) Hamano T., Nishi M., Itoh T., Ebihara S., Watanabe Y., *Oyo Yakuri-Pharmacometrics*, **72**, 31—38 (2007).
26) Singh A. K., Jiang Y., Benlhabib E., Gupta S., *J. Med. Food.*, **10**, 526—542 (2007).