Particle Size Modification of Curcumin and Its Effect on Plasma and Tissue Distribution

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Abstract

Curcumin is a polyphenolic compound with wide pharmacological activity, including anti-inflammatory, antioxidant, reno-protective, hepatoprotective, and anti-cancer properties. Despite its known benefits, use of curcumin is limited because of its poor absorption, bioavailability, and rapid elimination.

The aim of this study was to determine the effect of particle size modification on the concentration of curcumin in the plasma and tissues.

Nanocurcumin was produced from curcumin using the top-down method. Subsequently, 5 Sprague–Dawley rats per group were randomized to receive curcumin or nanocurcumin in a single oral dose (500mg/kg of body weight). Plasma and tissues (liver, kidney and colon) samples were obtained 180 and 240 min, respectively, after administration. Curcumin levels in the plasma and tissues were analyzed using UPLC-MS/MS. The obtained particle size of nanocurcumin was<100nm. Curcumin and nanocurcumin were detectable and measurable in the plasma, liver, kidney, and colon.

Overall, there were no significant differences in the concentration of curcumin after a single administration of curcumin or nanocurcumin in the plasma and liver, kidney, and colon tissues. Reduction of the particle size of curcumin to nanosize does not increase the concentration of curcumin in the plasma, colon, liver, and kidney tissues.

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Introduction

Curcumin is an active phytochemical component of the turmeric (*Curcuma longa*) plant, which is widely used in Asian countries as a dietary spice and traditional herbal medicine to treat a variety of diseases.[1]It has been studied extensively for its anti-inflammatory, anti-angiogenic, antioxidant, renoprotective, hepatoprotective, wound healing, and anti-cancer properties.[2–5]

However, despite its medicinal properties, the use of this drug in clinical practice is limited.[6] Curcumin has demonstrated negligible distribution to target tissues beyond the gastrointestinal tract, with low systemic bioavailability following oral dosing. Studies have shown that following its administration at a dose of 1g/kg of body weight, >75% of the curcumin was excreted in the feces, with negligible concentrations of curcumin detected in the urine. Measurement of curcumin concentrations in the plasma has shown that it was poorly absorbed in the gut. In addition to its poor absorption, the concentrations of curcumin reaching tissues outside the gut were pharmacologically inadequate.[7] Curcumin is insoluble in water at physiological pH and exhibits a rapid elimination profile.[8]

Numerous studies have been conducted to overcome these limitations by enhancing the solubility, absorption, bioavailability, and stability of curcumin. Particle size is one of the factors affecting drug absorption. Therefore, nanoparticle-based drug delivery may be a promising alternative.[9] Nanoparticle technology has been widely utilized to improve the distribution and cellular uptake of hydrophobic

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drugs.[10]The particle size of curcumin may be directly reduced or encapsulated into liposomes, solid lipid nanoparticles, or incorporated in complexes.[10–12] Therefore, the objective of the present study was to compare the concentrations of curcumin in the plasma and several tissues following a reduction of its particle size.

Materials and methods

Unmodified curcumin and nanocurcumin

Curcumin was obtained from Plamed Green Science Ltd. (China). The purity of the product was 99.0%, as stated in the Certificate of Analysis. Nanocurcumin was produced using the top-down (ball-milling) method in PT Nanotech Herbal Indonesia.

Measurement of curcumin and nanocurcumin particle size

The diameters of unmodified curcumin and nanocurcumin were determined using Delsa™ Nano.

Administration of curcumin and nanocurcumin in rats

Sprague–Dawley rats weighing 180–260g were placed in a room with constant temperature, humidity, and adequate illumination. Food (pellets) and water were provided *ad libitum*. All animal experiments were approved by the Ethical Committee of the Faculty of Medicine, Universitas Indonesia. The animals were treated according to the guidelines of animal experiments in the University.

The rats were fasted for 10h prior to the experiment, with access to water. Animals were randomly divided into 2 groups (5 rats per group) to orally receive 500mg/kg of body weight of unmodified curcumin or nanocurcumin. The dose was based on previous study by Siviero et al[13], Sample size was not calculated, however the maximum number of rats per group used in the study permitted by Ethics Committee of the University was 5. Carboxymethylcellulose were used as the vehicle to administer curcumin or nanocurcumin. Three hours after administration, blood samples were collected from the tail vein using EDTA tubes. Subsequently, the animals were decapitated 4h after administration to ensure that the drug was distributed to the tissues. Tissue samples were subsequently collected from the colons, kidneys, and livers of the sacrificed rats.

Sample preparation

Blood samples were centrifuged at 3000 rpm for 10 min to obtain plasma. Curcumin concentration analysis was performed using UPLC-MSMS. Tissues from the colons, livers, and kidneys were rinsed with saline solution, dried on filter paper, and weighted. Homogenates were produced by homogenization of 100mg of colon, liver, or kidney tissue in 1 mL saline solution, centrifugation at 12000 rpm, collection of the supernatant, and transfer to tubes for curcumin concentration analysis.

Sample processing

Propranolol (20µL) (internal standard) was added to 200µL of plasma or tissue homogenates and. The mixture was then further extracted using methyl tert-buthyl ether/MTBE (Sigma-Aldrich, Singapore). The obtained organic layer was subsequently evaporated using a nitrogen stream. The residue was dissolved in the mobile phase and transferred to vials for analysis using UPLC-MS/MS.

Analysis of the concentration of curcumin in plasma and tissue homogenates using UPLC-MS/MS

The analysis of the concentrations of curcumin in plasma and tissue homogenates was performed using UPLC-MS/MS (Waters™). Analytes were monitored at m/z of 269.25 → 177.08 for curcumin and 260.06 → 183.17 for propranolol (internal standard). Samples were separated using a C18 column (2.1 × 50 mm, 1.7µm) and a gradient mobile phase of 0.1% formic acid and acetonitrile (78:32). The concentration of curcumin in the plasma and tissues was calculated using a calibration curve of the curcumin standard.

Statistical analysis

The results are represented as mean±standard error of the mean. Statistical analyses were performed using Student's t-test to determine differences between groups. The level of significance was set at $p<0.05$.

Results

The average particle sizes of curcumin and nanocurcumin were 331 and 82 nm, respectively (Figure 1). Three hours after administration, the concentration of curcumin in the plasma was analyzed.

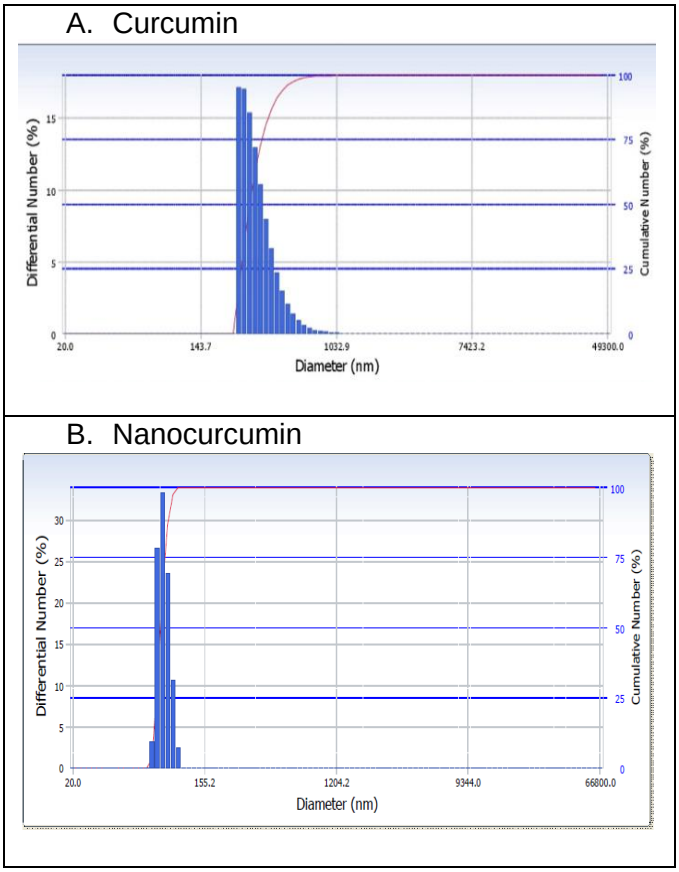

Figure 1. Particle size distribution of (A) curcumin and (B) nanocurcumin.

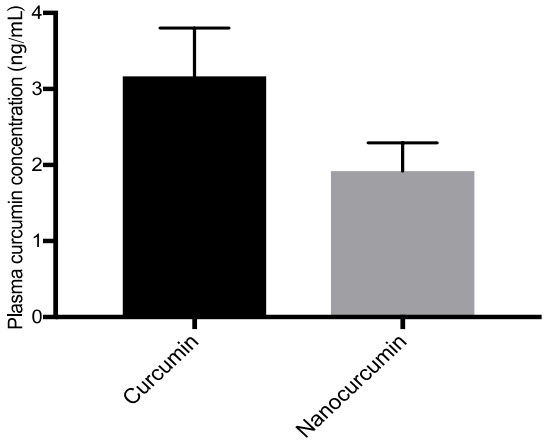

Figure 2. Concentration of curcumin in rat plasma 180 min after a single oral administration of curcumin or nanocurcumin.

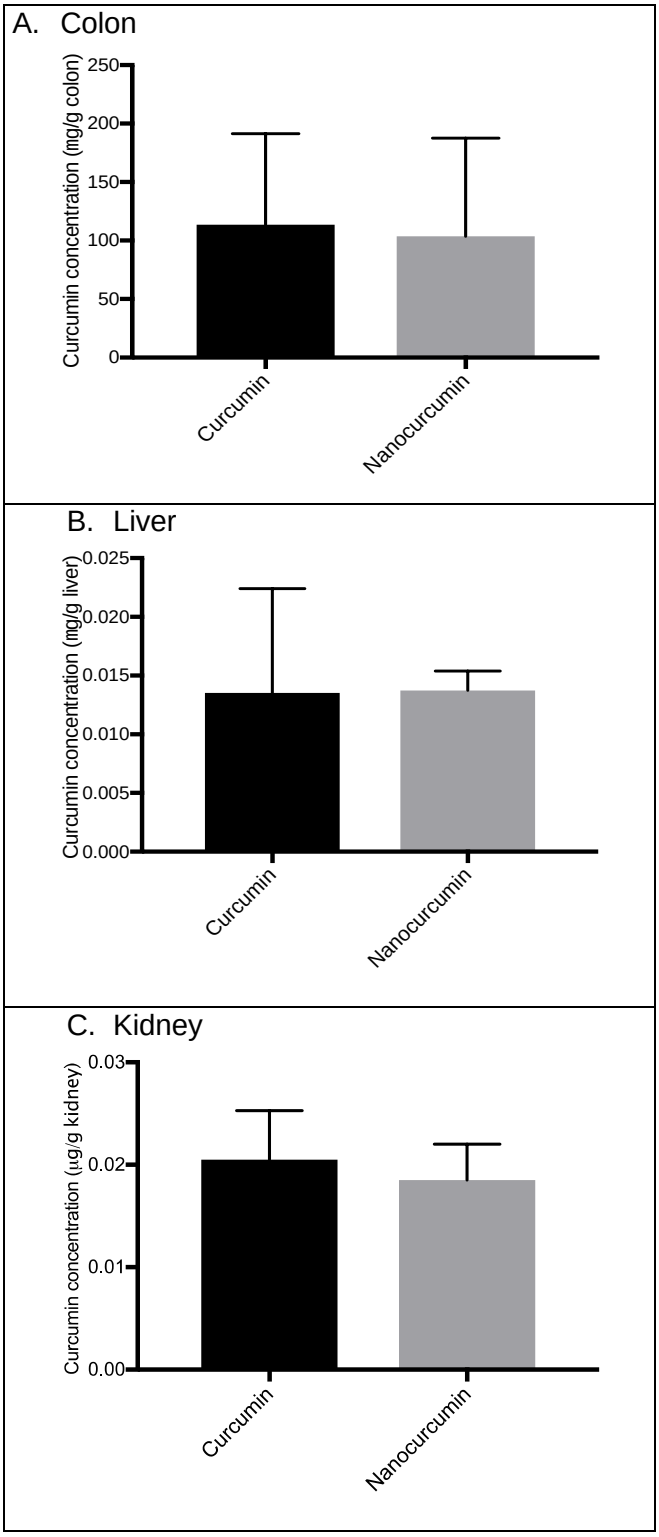

Figure 3. Concentration of curcumin in (A) colon, (B) liver, and (C) kidney tissues 240 min after a single oral administration of curcumin or nanocurcumin.

A slightly lower concentration of curcumin was detected in the plasma after the administration of nanocurcumin compared with

that measured after the administration of unmodified curcumin (Figure 2). However, the observed difference was not statistically significant (p=0.31).

Moreover, in the colon, liver, and kidney tissues, there were no differences observed in the concentration of curcumin 240 min after administration of unmodified curcumin or nanocurcumin (Figure 3).

Discussion

Numerous studies have suggested the use of curcumin in various indications, due its excellent pharmacological profile.[1] However, its clinical application is limited due to its low bioavailability and tissue distribution profile. Unmodified curcumin exhibits poor solubility in water, low permeability through the intestinal mucosa, easy degradation in neutral or acidic pH, and rapid elimination.[8] Several approaches have been investigated to overcome this problem, one of which is the modification of the particle size of curcumin into nanosize.

In the present study, we reduced the particle size of curcumin from 331 to 82 nm using the top-down method. It was hypothesized that the nanocurcumin would exhibit an improved absorption profile and, consequently, increase its concentration in tissues (particularly the liver and kidney). However, in contrast to our hypothesis, we did not observe significant differences in the concentration of curcumin in the plasma, as well as in the colon, liver, and kidney tissues. Several factors may be responsible for this observation. Nanoparticles have been reported to penetrate across intestinal epithelium through either transcellular or paracellular transport to enhance their absorption in the gastrointestinal tract. Nanosized particles may undergo transcellular transport through the epithelial cells of the small intestine via caveolae- and clathrin-mediated endocytosis, or caveolae- and clathrin-independent endocytosis.[14] Nanoparticles internalized in epithelial cells may be translocated to the endolysosome, where they remain intact and enter the blood circulation through exocytosis.[15] Otherwise, they may be rapidly degraded in the matured endolysosomes after clathrin-mediated endocytosis.[16] Owing to their different particle sizes, in the present study, nanocurcumin may have been transported across intestinal cells through a similar mechanism, whereas curcumin accumulated intracellularly. Therefore, it is likely that penetration across the intestinal epithelium into the circulation may reduce the amount of nanocurcumin directly absorbed by the epithelial cells of the rat colon.

Wahlang et al.[17] demonstrated that curcumin exhibits higher permeability in the apical-to-basolateral direction of the cell compared with that observed in the opposite direction. This evidence indicates that curcumin may not be a substrate for active transport. Moreover, the intracellular accumulation of curcumin within the cells has also been revealed through confocal scanning laser microscope.[17]

As nanoparticles enter the gastrointestinal tract, they are exposed to different pH, excess amounts of ions, and different kinds of digestive enzymes. In addition, reduction of curcumin to tetrahydrocurcumin by intestinal bacteria has been reported.[18] Owing to its smaller particle size, nanocurcumin provides a higher surface area-to-volume ratio available for enzymatic reaction by intestinal bacteria.

Prior to reaching the circulatory system, curcumin undergoes the hepatic first-pass effect, and this metabolic process yields glucoronide and sulfate conjugates. During entry into the enterohepatic circulation, a large quantity of curcumin and its conjugates is excreted in the bile. Furthermore, trace levels of curcumin may also be detected in the urine via excretion through the kidneys. A previous study showed that 50% of the curcumin dose was excreted in the bile within 5 h after intravenous administration, implying that curcumin undergoes biotransformation during absorption in the intestinal tract and enterohepatic recirculation.[19] Hence, considering the extensive metabolism in the intestine and liver along with the rapid excretion rate, the distribution of nanocurcumin in tissues may be limited. Moreover, its quantity available for direct absorption into the rat colon is further declined.

In addition, the mucus layer lubricating the epithelial surfaces serves as a protective physical barrier. The translocation of particles depends on their efficiency to diffuse through the mucus and the membrane. This efficiency is affected by various factors, such as particle size and surface charge. Smaller particles are transported faster through the membrane into the circulatory system than larger particles.

Furthermore, positively charged particles penetrate faster through the mucus layer because of its electrostatic interactions with negatively charged mucin. Shaikh et al.[19] corroborated this finding, demonstrating that nanoformulation of curcumin leads to a 10-fold increase in its concentration in the plasma versus that of unmodified curcumin.[19] In curcumin nano-formulation, nanoparticles are encapsulated with positively charged polymers or surfactant biodegradable copolymers with hydrophilic segments. This process increases the permeability and solubility of the drug and maintains a high stability of the nanoparticulate system in biological milieu.

However, in the present study, curcumin nanoparticles were not nano-formulated. Hence, both curcumin and nanocurcumin exhibit an identical electrical neutrality. The lack of a difference between the mean concentrations of curcumin and nanocurcumin suggests that they may be anchored in the mucus, lowering the cellular uptake of particles by epithelial cells.[20] Behren et al. provided evidence of hydrophobic nanoparticle entrapment in the mucus, leading to lower association with epithelial cells.[21]

Besides its poor metabolic stability, curcumin also displays poor chemical stability. Of note, its stability in the gastrointestinal tract is highly affected by pH.[22] As stated earlier, a decrease in particle size indicates a higher surface area-to-volume ratio. Therefore, nanocurcumin is more likely to be chemically degraded in the gastrointestinal tract versus curcumin.

Large quantities of ions are present in the gastrointestinal tract to regulate the pH, signal the transport of nutrient compounds, and activate enzymatic processes in the digestive tract. Nanoparticles and ions interact because of ionic attraction, thus increasing the nanoparticle size. Ions bind with water via dipole attraction. It is established that the presence of excess ions in the gastrointestinal tract may cause nanoparticle aggregation or precipitation, consequently reducing the hydration of nanoparticles in water.[23] We deduce that the formation of aggregates of nanocurcumin may explain the lower concentration of nanocurcumin compared with that observed for curcumin in the colon of rats. With increased particle size, these nanocurcumin crystals demonstrate reduced solubility, resulting in poorer absorption in the colon versus that observed for curcumin.

Conclusions

In conclusion, reduction of the particle size of curcumin using top-down (ball-milling) method does not result in increased concentration of curcumin in the plasma and tissues.

Declaration of Interest

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