Journal of the American Association for Laboratory Animal Science
Vol 57, No 5
September 2018
Pages 508–512
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Administering Fixed Oral Doses of Curcumin to Rats through Voluntary Consumption

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Curcumin, a polyphenol derived from turmeric, has a wide variety of therapeutic benefits including antiinflammatory, antioxidative, and chemopreventative effects. Oral gavage is widely performed to administer curcumin in laboratory rodents in several experimental models. Although effective, this method can increase stress in the animal, potentially influencing experimental results. Moreover, oral gavage can result in mortality due to accidental instillation of fluid into the lungs, serious mechanical damage, and gavage-related reflux. Here we describe a method for the administration of fixed dosages of curcumin to rats through voluntary consumption of peanut butter, to reduce gavage-related morbidity and distress to animals and to provide environmental enrichment. Fischer 344 (*n* = 6) rats received 1100 mg/kg of a commercial curcumin product (equivalent to approximately 200 mg/kg of curcumin) in 8 g/kg of peanut butter daily for 5 wk. Curcumin concentrations in rat plasma were measured by using UPLC–MS at 2 to 4 h after administration. All rats voluntarily consumed the peanut butter–curcumin mixture consistently over the 5-wk period. Total curcumin concentrations in plasma samples collected 2 to 4 h after curcumin consumption were 171 ± 48.4 ng/mL (mean ± 1 SD; range, 103 to 240 ng/mL). This noninvasive curcumin delivery method was effective, eliminated the stress caused by daily oral gavage, and added environmental enrichment.

Abbreviation: PB, peanut butter

DOI: 10.30802/AALAS-JAALAS-17-000143

Curcumin (diferuloylmethane; 1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) is a polyphenol derived from turmeric, the rhizome of the plant *Curcuma longa*. Curcumin is both the primary biologically active component of turmeric and responsible for its yellow pigmentation. Curcumin has been used for centuries in traditional Chinese and Indian medicines to treat various illnesses, including ulcers, liver disease, wounds, and the common cold. Over the last few decades, curcumin has been studied more extensively in modern medicine, due to its pleotropic nature, low toxicity, and tolerability.[1] Curcumin possesses both antioxidant and antiinflammatory activities and has been studied as a potential therapeutic in neurologic disorders, such as Parkinson disease, Alzheimer disease, and depression.[13,18,24] Curcumin has been used effectively as an anticancer agent in a wide variety of tumors, in which it stimulates apoptosis (BCL2, BCL-XL, and BAX), downregulates proproliferative pathways (PI3k, NFκB, JAK–STAT, MAPK, TGFβ, Wnt-β–catenin, and sonic hedgehog), and reduces angiogenesis.[1]

Orally administrated curcumin is well tolerated, even at extremely high doses, in both animals and humans.[7,25] For oral administration to laboratory animals, fixed doses of curcumin typically are administered through oral gavage. This procedure involves the passage of a rigid dosing cannula or flexible plastic tubing through the esophagus and into the stomach while the animal is manually restrained. Although effective, oral gavage can result in death of experimental animals due to accidental dosage into the lung, perforation of esophagus or stomach, or gavage-related reflux.[10] In addition, repeated oral gavage can lead to animal distress, which is known to elicit a variety of biochemical and physiologic changes, including adult neurogenesis, and to affect metabolism, heart rate, endocrine function, and inflammatory processes.[3] This gavage-related stress may alter experimental results, particularly when stress affects the physiologic or pathophysiologic process that is studied.

Alternatively, curcumin can be ingested through voluntary consumption by adding it to dry feed; however, custom-formulated feed is expensive, and the dose that each animal actually consumes is highly variable.[20] Peanut butter (PB) has effectively been used as a vehicle for voluntary consumption of losartan in rats.[11] Here we describe a method for the administration of fixed dosages of curcumin in PB through voluntary consumption to reduce gavage-related morbidity in and distress to animals. We conducted the current study to demonstrate that voluntary ingestion of curcumin in PB by rats is feasible in principle as an alternative to oral gavage over prolonged periods of time.

Materials and Methods

Animals. Male and female Fischer 344 rats (age, 12 wk; Flinders University School of Medicine Animal Facility) were housed 3 per cage (31 cm × 38 cm × 38 cm; GR1800 double-decker rat cages, Tecniplast, Buguggiate, Italy). Cages contained recycled paper pellets, (chemical- and additive-free, Back-2-Nature Animal Bedding, Fibrecycle, Queensland, Australia), shredded paper, and cardboard rolls or boxes for environmental enrichment. Animals were housed in a temperature-controlled (22 ± 1 °C) and humidity-controlled (60% ± 5%) environment on a 12:12-h light:dark cycle. Rats had free access to food (Gordon's Autoclavable Premium Rat and Mouse Pellets, Gordon's Specialty Stock Feed, New South Wales, Australia) and water. Approval was gained from the Flinders University and Southern Adelaide Local Health Network Animal Welfare Committee

Received: 07 Nov 2017. Revision requested: 12 Jan 2018. Accepted: 22 Mar 2018.
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(approval no. 892/15) in accordance with the State Government of South Australia Animal Welfare Act, 1985, and the National Health and Medical Research Council Australian Code for the Care and Use of Animals for Scientific Purposes, 2013. Animals were housed in a pathogen-controlled facility.

Palatability of curcumin–PB mix. When given the choice, rats preferred PB (100% natural smooth peanut butter, Sanitarium, New South Wales, Australia) over hazelnut–chocolate spread (data not shown); we therefore chose PB as the vehicle for the administration of a commercial curcumin product (phospholipid-curcumin complex, Meriva Bioavailable Curcumin, Indena, Milan, Italy). To acclimate rats to the taste of PB, they received 8 g/kg of PB alone for 3 d prior to receiving the curcumin–PB mixture. The PB was given after 3 h of fasting at the beginning of the lights-on phase of the light:dark cycle, and rats were given 2 h to consume the PB. Appropriate amounts of PB were weighed into Dacron bowls (Biomedical Engineering, Flinders Medical Centre, South Australia, Australia) and attached to the side of the cage by using adhesive tape. Bowls were designed to be durable, autoclavable, and readily accessible, to allow rats to completely consume the doses (Figure 1). The bowls were attached to the side of the cage to ensure that rats would not walk through the bowl and contaminate the curcumin–PB mixture with bedding materials or waste. Rat were placed in individual cages so that consumption could be monitored. The curcumin–PB mixture used in this study was determined by testing the consistency of the curcumin product in different ratios of peanut butter. A dose of 1100 mg/kg of curcumin product (equivalent to approximately 200 mg/kg of curcumin) in 8 g/kg of PB was chosen as the optimal dose according to consistency. To test the palatability of the curcumin–PB mixture Rats were given increasing amounts of the curcumin product in 8 g/kg of PB. Rats received 275 mg /kg of the curcumin product in 8 g/kg PB for 2 consecutive days, 550 mg/kg in 8 g/kg for 2 consecutive days, and the full dose thereafter.

Curcumin administration in rats through oral consumption. Fischer 344 rats (3 male, 3 female) were fasted for 3 h prior to receiving 1100 mg/kg of curcumin product in 8 g/kg of PB. The curcumin product was thoroughly integrated into the PB manually, by using a microspatula, immediately before each dose was given. Appropriate doss were weighed into Dacron bowls and attached to the side of the cage by using adhesive tape. Rats were housed in separate cages (28 cm × 41 cm × 25 cm) and received the curcumin–PB mixture daily for 5 wk after a 3-h fasting period at the beginning of the light-on phase of the photocycle. The bowls used to provide the curcumin–PB mixture were visually examined and weighed before and after consumption to ensure that the full dose had been ingested. Animal weight was monitored weekly throughout the experiment. Unpaired *t* tests were used to compare the weights of the study population with a cohort of historical control animals using GraphPad Prism 7 Software (San Diego, CA). A P value less than 0.05 was considered statistically significant.

Plasma collection. Blood was collected weekly from the tail vein of rats, at 2, 3, and 4 h after consumption of the curcumin–PB mixture. Topical anesthetic cream (4% lidocaine w/w, LMX4) was applied to the tail of rats 15 min prior to blood collection. Rats were secured in a restrainer (Flinders University, Biomedical engineering) and placed in a 35 °C incubator for 15 min to prompt vasodilation of the tail vein. Blood was collected into microcuvettes (Microvette 200 µL, Lithium Heparin, Sarstedt, Newton, NC) by using a 23-gauge needle. Samples were centrifuged for 5 min at 2000 × *g*, and plasma was transferred to a fresh tube. Fecal samples were collected 24 h after curcumin administration. After 5 wk of daily oral curcumin–PB, rats were euthanized by CO_2 induction after isoflurane anesthesia (4%). A final blood sample was collected into heparinized tubes (4 mL, Lithium Heparin Blood Collection Tubes, Sarstedt) by cardiocentesis at 2 to 4 h after consumption of the curcumin–PB mixture. All plasma samples were stored at –80 °C until analysis was performed.

Enzymatic hydrolysis of curcumin conjugates from plasma samples. Enzymatic hydrolysis of curcumin conjugates was performed by using β-glucuronidase and sulfatase as previously described.[2,22] Plasma samples (200 µL) were diluted in 80 µL of water, 50 µL of β-glucuronidase (446 units) in 0.1 M sodium phosphate buffer (pH 6.8), and 45 µL of sulfatase (52 units) in 0.1 M sodium acetate buffer (pH 5.0) and incubated for 3.5 h at 37 °C. At the completion of the incubation, curumin-d6 (10 µL, 8 µg/mL) was spiked into the samples as an internal standard for the assay. Fecal samples were weighed and diluted in 1:10 in PBS. Diluted fecal samples (200 µL) were diluted in 80 µL of water, 50 µL of 0.1 M sodium phosphate buffer (pH 6.8), and 45 µL of 0.1 M sodium acetate buffer (pH 5.0); curumin-d6 (10 µL, 8 µg/mL) was spiked into the samples as an internal standard for the assay.

Extraction of curcumin from plasma and fecal samples. The extraction method was performed as previously described.[22] Samples were mixed with 1 mL of extraction buffer (ethyl acetate:methanol, 95:5 [v/v]) and vortexed for 30 s. The upper solvent and lower aqueous phases were left to separate on standing for 10 min at room temperature. The lower aqueous layer was frozen in an ethanol–dry-ice bath, and then the upper solvent layer was decanted into a clean tube. Extraction of the lower aqueous phase was repeated twice, for a total of 3 extractions, and the pooled solvent extracts were evaporated to dryness on a vacuum concentrator (miVac Duo Concentrator, SP Scientific, Warminster, PA) for 30 min at 40 °C. The extracts were reconstituted in 100 µL of methanol, and 5 µL was analyzed by UPLC–mass spectrometry.

Quantitation of curcumin in plasma and fecal samples. Curcumin analysis was performed on a UPLC system (ACQUITY, Waters, Milford, MA) coupled to a quadrapole time-of-flight mass spectrometer (Premier, Waters) with an electrospray ionization source operated in negative ionization mode. Time-of-flight data were collected in MS mode between 100 and 1000 Da by using an instrument scan time of 1 s and interscan delay of 0.02 s. The experimental parameters were: capillary voltage, 3.0 kV; source temperature, 100 °C; desolvation temperature, 300 °C; sampling cone voltage, 30 eV; and extraction cone voltage, 5 eV. The collision gas flow was 0.5 mL/min. Instrument control, data acquisition, and data processing were performed by using MassLynx version 4.1 software (Waters). In addition, the UV–visible chromatogram was recorded at 420 nm.

Chromatographic separation was performed at a flow rate of 0.3 mL/min on a C18 column (1.7 µm, 2.1 mm × 100 mm; ACQUITY UPLC BEH C18 column, Waters) held at 35 °C. Mobile phase composition was 10% v/v acetonitrile in water (mobile phase A) and acetonitrile (mobile phase B). Initial conditions were 70% mobile phase A and 30% mobile phase B. The proportion of mobile phase B was increased linearly to 60% over 5 min and then returned to 30% for 2 min to reestablish equilibrium before injection of the next sample for analysis.

Extracted ion chromatograms were obtained with a mass window of 0.02 Da from total ion chromatograms using the m/z value corresponding to the monoisotopic mass of curcumin ($[M-H]^-$ = 367.13 amu) and for curcumin-d6 as an internal standard ($[M-H]^-$ = 373.16 amu) . For quantitation, human plasma with no detectable curcumin (190 µL) was spiked with a standard solution of curcumin in DMSO (10 µL) to yield final curcumin

Figure 1. Bowls used for curcumin–PB dosing in Fisher 344 rats. (A) Rat consuming curcumin–PB mixture from a bowl attached to the side of the animal enclosure. (B) Bowls without and with curcumin–PB mixture.

concentrations of 0, 0.5, 1, 1.5 and 2 µg/mL; these samples then were extracted and reconstituted in the same manner as incubation samples. The calibration curve was constructed by plotting the peak area ratio of curcumin to internal standard compared with the curcumin concentration.

Results

Palatability of curcumin–PB mix. On days 1 and 2 of the acclimation period, some of the rats did not consume the full dose of PB containing the lower doses of the curcumin product, but by day 3, all rats consumed the entire dose of PB within the allocated 2 h. After the acclimation period, all rats readily consumed all of the doses of curcumin–PB mixture.

Voluntary consumption of curcumin–PB mix. After a 3-h fast, Fischer 344 rats voluntarily and consistently consumed the curcumin–PB mixture daily for a 5-wk period. All rats adapted without any difficulties to daily consumption of the curcumin–PB mixture and consumed the entire dose within 5 to 30 min (Figure 1). Consumption time appeared to decrease as the rats became more acclimated to the taste of the curcumin-PB mixture, although precise consumption times were not measured during this experiment. The rats required 30 min to consume the curcumin–PB dose at the beginning of the experiment but only 5 to 10 min at study end. No adverse effects on animal wellbeing, such as porphyrin build up around the eyes, were observed, and the rats appeared enthusiastic about receiving the curcumin–PB mixture. The weight of rats that ingested the curcumin–PB mixture was comparable to those of the same age and line and fed the same standard diet (Figure 2). Total weight gain over 5 wk did not differ between both male and female curcumin–PB-treated rats and historical controls (P = 0.7381 and P = 0.4871, respectively). We observed remnants of curcumin–PB mixture in the stomach of rats culled at 2 to 4 h after consumption.

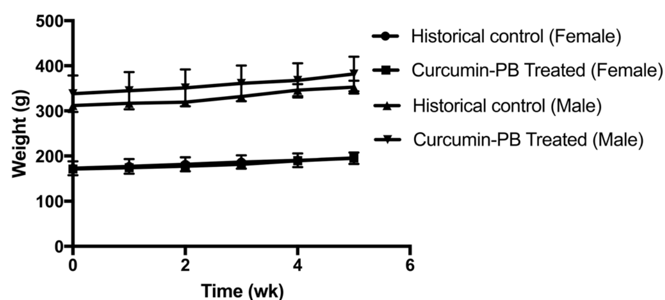

Figure 2. The weights of rats that ingested the curcumin–PB mix daily for 5 wk was comparable to historical controls of similar age.

Curcumin levels in plasma. After β-glucuronidase and sulfatase deconjugation, total curcumin was detected in the plasma. The concentration of curcumin (mean ± 1 SD) in plasma collected between 2 to 4 h after curcumin consumption was 170.7 ± 45.1 ng/mL (range, 100 to 240; Table 1). Curcumin was detected consistently in the plasma of rats throughout the 5-wk period. In addition, curcumin plasma concentrations were similar among samples collected at 2, 3, and 4 h after consumption (Table 1).

Curcumin levels in feces. Free curcumin was detected at high concentrations in rat fecal matter. The curcumin concentration

Table 1. Plasma total curcumin concentration (ng/mL) after administration of curcumin–PB (200 mg/kg) to Fisher 344 rats

Time (h)	Individual curcumin concentrations	Group mean concentration	Overall concentration (mean ± 1 SD)
2	103, 120, 179, 210, 211, 220	174	170.7 ± 45.1
3	160, 142, 171	158	
4	118, 174, 240	177	

The 2-h group contained 3 female and 3 male rats; the 3- and 4-h groups each had 2 female and 1 male rats.

(mean ± 1 SD; n = 3) in fecal samples collected at 24 h after consumption was 1897 ± 299 µg/g.

Discussion

Here we describe a method for daily delivery of fixed doses of curcumin to rats through voluntary oral consumption in PB that could be applied to other experimental compounds. Oral delivery is commonly used as the route of administration of curcumin, in both human and animal studies. In animal studies, oral gavage is used most frequently to deliver a precise dose of curcumin. Because curcumin is highly insoluble in aqueous solutions, it is usually administered orally as a suspension in various oils (peanut, corn, corn oil, or sunflower oil), or in carboxymethylcellulose.[5,6,21] Extensive training is required to effectively and safely administer substances through oral gavage. Although oral gavage can be effective when performed by skilled personnel on rats acclimated to handling, it can lead to the premature death of the test animals either by accidental administration of fluid into the lungs, serious mechanical damage to esophagus or stomach, or gavage-related reflux.[4,10] In addition, the procedure may require 2 operators because of difficulties in single-handed restraint, depending on the size or temperament of the animal. Previously, we found that rats could be acclimated to the oral gavage procedure over 1 wk by using water or dilute fruit juice, but they rapidly developed a taste aversion to the curcumin and displayed distress behaviors, including struggling against manual restraint, biting the plastic gavage tubing, vocalization, and diarrhea. This problem was exacerbated by the difficulty of administering curcumin solution through oral gavage tubing due to the viscosity of the vehicle and insolubility of curcumin. As a result, the oral gavage tube often needed to be inserted for prolonged periods of time or repeatedly. In addition, blockage of syringes due to insoluble curcumin might require multiple reinsertions of the tubing to effectively deliver the entire dose, and we often noted insoluble curcumin left in the hub of the needle, resulting in inaccurate dosing.

Mixing the curcumin dose with PB is a cost-effective and reliable alternative to specialized diets and allows a fixed dose of curcumin to be given to each animal at specified times by a single person. Flavored gelatin, various nut pastes, syrup, sugar dough, bacon-flavored dough, and cookie dough have all been used for voluntary administration of various drugs in laboratory rodents.[8,11,23] Curcumin is highly lipophilic; therefore we hypothesize that administering curcumin in a high-fat compound such as PB would not negatively affect curcumin absorption. In addition, PB effectively disguises the bitter, earthy taste of curcumin, making it more palatable to the rats. Furthermore, PB is a good source of dietary protein and fats, offers additional environmental enrichment, and can be purchased in 'pure' forms that lack additives such as salt, sugar, palm oil, and stabilizers.

Animals can experience stress in response to oral gavage and manual restraint. Given that stress causes a wide range of behavioral, biochemical, and physiologic changes, this response is an important consideration in experiment design, particularly when stress affects the pathophysiologic process under investigation. Corticotropin-releasing hormone is secreted from the hypothalamus in response to stressors, resulting in the secretion of corticotropin from the anterior pituitary, which in turn stimulates the release of glucocorticoids such as corticosterone from the adrenal gland.[19] Glucocorticoids provoke multiple changes, including the activation of gluconeogenesis, increased hepatic protein synthesis, and downregulation of the inflammatory response. In addition, activation of the stress response leads to increased heart rate and blood pressure as well as changes in gastric secretions and gut motility.[19] Corticosterone levels in rodents are increased after oral gavage.[3] Mice often demonstrate significant elevations in plasma corticosterone levels after oral gavage of various oils; these levels are similar to those detected in mice demonstrating stress-induced hyperthermia.[12]

Curcumin is poorly absorbed into the circulation through the intestinal mucosa, where it undergoes enzyme-mediated biotransformation.[17] Once taken up by intestinal enterocytes, curcumin undergoes enzymatic modification (phase I metabolism) and conjugation (phase II metabolism) by oxidoreductases, sulfotransfereases, and glucuronosyltranferases.[14] Many different curcumin formulations and drug-delivery systems have been developed to improve the compound's stability, solubility, and bioavailability in vivo; these include polymer-based vehicles such as nanoparticle-formulated curcumin, molecular-complexed curcumin (curcumin–plasma protein), and lipid-based vehicles such as curcumin-loaded micelles.[15] In the current study, we used a phosphatidylcholine formulation of curcumin that has been tested in clinical trials and approved by the Therapeutic Goods Administration. This product is reportedly 29 times more bioavailable than standard unformulated curcumin.[9] Consistently, low plasma concentrations of curcumin are reported, even after extremely high oral doses. One study reported a plasma concentration of only 16.1± 11.4 ng/mL at 20 min after administration of a dose equivalent to 340 mg/kg by oral gavage.[16] Low concentrations in the systemic circulation are due to a combination of factors, including low oral bioavailability, low solubility, poor intestinal absorption, and rapid metabolism. Curcumin is primarily metabolized into curcumin- O-glucuronide and curcumin-O-sulfate after oral administration.[14] Because we were unable to detect free curcumin in plasma, we measured the concentration of 'total curcumin' in plasma and tissue after deconjugation by β-glucuronidase and sulfatase enzymes. The plasma concentrations we obtained (170.6 ± 44.1 ng/mL) were consistent across the 2-, 3-, and 4-h time points, suggesting that curcumin was slowly absorbed into the bloodstream over hours rather than minutes. This effect is likely due to prolonged gastric emptying due to ingestion of PB, which is high in saturated and unsaturated fats. In support of this hypothesis, we noted curcumin–PB mixture in the stomachs of rats at 2, 3, and 4 h after consumption. In addition, PB may decrease the amount of curcumin exposed to enzymatic degradation. Large amounts of free curcumin were present in the feces of rats after oral consumption, which is in keeping with

a previous study that observed that 40% of free curcumin was excreted unchanged in the feces.[17]

Voluntary oral consumption of curcumin–PB mixtures may be unsuitable when studying models in which appetite is suppressed and incomplete ingestion is likely. In this case, oral gavage may be required to administer a fixed dose of curcumin to test animals. We needed to use 1 to 3 g of PB to disguise the taste of the large dose of the curcumin product (1100 mg/kg; equivalent to 200 mg/kg curcumin). This dose may alter lipid profiles, and this effect should be considered when using this delivery method in various animal models. The amount of PB needed to give a fixed dose of curcumin might be decreased if administering pure curcumin at 200 mg/kg or lower doses. We implemented the 3-h fast to increase the rats' hunger prior to receiving curcumin-PB mix. The fasting period occurred during the beginning of the lights-on phase of the animal's light:dark cycle, during which rats consume little food. Therefore this step potentially could be omitted from the protocol without affecting the consumption of the curcumin-PB mixture, but this modification would have to be tested. In the current study, rats were acclimated to the taste of PB for 3 d before they were slowly introduced to the curcumin–PB mixture. This acclimation period was critical to compliance with voluntary consumption of curcumin–PB mixture. The acclimation period could be increased to decrease consumption time early during the experiment.

In conclusion, we here described a method suitable for daily, long-term voluntary oral consumption of precise doses of curcumin in peanut butter that was palatable for rodents. This noninvasive curcumin delivery method eliminates the stress caused by daily manual animal capture and oral gavage and the potential for gavage-related injury and provides environmental enrichment.

References

1. **Allegra A, Innao V, Russo S, Gerace D, Alonci A, Musolino C.** 2016. Anticancer activity of curcumin and its analogues: preclinical and clinical studies. Cancer Invest **35:**1–22. https://doi.org/10.1080/07357907.2016.1247166.
2. **Asai A, Miyazawa T.** 2000. Occurrence of orally administered curcuminoid as glucuronide and glucuronide/sulfate conjugates in rat plasma. Life Sci **67:**2785–2793. https://doi.org/10.1016/S0024-3205(00)00868-7.
3. **Bonnichsen M, Dragsted N, Hansen A.** 2005. The welfare impact of gavaging laboratory rates. Anim Welf **14:**223–227.
4. **Brown AP, Dinger N, Levine BS.** 2000. Stress produced by gavage administration in the rat. Contemp Top Lab Anim Sci **39:**17–21.
5. **Carroll CE, Benakanakere I, Besch-Williford C, Ellersieck MR, Hyder SM.** 2010. Curcumin delays development of medroxyprogesterone acetate-accelerated 7,12-dimethylbenz[a]anthracene-induced mammary tumors. Menopause **17:**178–184. https://doi.org/10.1097/gme.0b013e3181afcce5.
6. **Chang MT, Tsai TR, Lee CY, Wei YS, Chen YJ, Chen CR, Tzen JT.** 2013. Elevating bioavailability of curcumin via encapsulation with a novel formulation of artificial oil bodies. J Agric Food Chem **61:**9666–9671.
7. **Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, Ko JY, Lin JT, Lin BR, Ming-Shiang W, Yu HS, Jee SH, Chen GS, Chen TM, Chen CA, Lai MK, Pu YS, Pan MH, Wang YJ, Tsai CC, Hsieh CY.** 2001. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Res **21:**2895–2900.
8. **Corbett A, McGowin A, Sieber S, Flannery T, Sibbitt B.** 2012. A method for reliable voluntary oral administration of fixed dosage (mg/kg) of chronic daily medication to rats. Lab Anim **46:**318–324. https://doi.org/10.1258/la.2012.012018.
9. **Cuomo J, Appendino G, Dern AS, Schneider E, McKinnon TP, Brown MJ, Togni S, Dixon BM.** 2011. Comparative absorption of a standardized curcuminoid mixture and its lecithin formulation. J Nat Prod **74:**664–669. https://doi.org/10.1021/np1007262.
10. **Damsch S, Eichenbaum G, Tonelli A, Lammens L, Van den Bulck K, Feyen B, Vandenberghe J, Megens A, Knight E, Kelley M.** 2011. Gavage-related reflux in rats: identification, pathogenesis, and toxicological implications (review). Toxicol Pathol **39:**348–360. https://doi.org/10.1177/0192623310388431.
11. **Diogo LN, Faustino IV, Afonso RA, Pereira SA, Monteiro EC, Santos AI.** 2015. Voluntary oral administration of losartan in rats. J Am Assoc Lab Anim Sci **54:**549–556.
12. **Gonzales C, Zaleska MM, Riddell DR, Atchison KP, Robshaw A, Zhou H, Sukoff Rizzo SJ.** 2014. Alternative method of oral administration by peanut butter pellet formulation results in target engagement of BACE1 and attenuation of gavage-induced stress responses in mice. Pharmacol Biochem Behav **126:**28–35. https://doi.org/10.1016/j.pbb.2014.08.010.
13. **Goozee KG, Shah TM, Sohrabi HR, Rainey-Smith SR, Brown B, Verdile G, Martins RN.** 2016. Examining the potential clinical value of curcumin in the prevention and diagnosis of Alzheimer's disease. Br J Nutr **115:**449–465. https://doi.org/10.1017/S0007114515004687.
14. **Heger M, van Golen RF, Broekgaarden M, Michel MC.** 2013. The molecular basis for the pharmacokinetics and pharmacodynamics of curcumin and its metabolites in relation to cancer. Pharmacol Rev **66:**222–307. https://doi.org/10.1124/pr.110.004044.
15. **Liu L, Sun L, Wu Q, Guo W, Li L, Chen Y, Li Y, Gong C, Qian Z, Wei Y.** 2013. Curcumin loaded polymeric micelles inhibit breast tumor growth and spontaneous pulmonary metastasis. Int J Pharm **443:**175–182. https://doi.org/10.1016/j.ijpharm.2012.12.032.
16. **Marczylo TH, Steward WP, Gescher AJ.** 2009. Rapid analysis of curcumin and curcumin metabolites in rat biomatrices using a novel ultraperformance liquid chromatography (UPLC) method. J Agric Food Chem **57:**797–803. https://doi.org/10.1021/jf803038f.
17. **Ravindranath V, Chandrasekhara N.** 1980. Absorption and tissue distribution of curcumin in rats. Toxicology **16:**259–265. https://doi.org/10.1016/0300-483X(80)90122-5.
18. **Tizabi Y, Hurley LL, Qualls Z, Akinfiresoye L.** 2014. Relevance of the anti-inflammatory properties of curcumin in neurodegenerative diseases and depression. Molecules **19:**20864–20879. https://doi.org/10.3390/molecules191220864.
19. **Tsigos C, Kyrou I, Kassi E, Chrousos GP.** 2016. Stress, endocrine physiology and pathophysiology. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, Koch C, Korbonits M, McLachlan R, New M, Purnell J, Rebar R, Singer F, Vinik A, editors. Endotext. South Dartmouth (MA): MDText.com.
20. **Turner PV, Brabb T, Pekow C, Vasbinder MA.** 2011. Administration of substances to laboratory animals: routes of administration and factors to consider. J Am Assoc Lab Anim Sci **50:**600–613.
21. **Ung VY, Foshaug RR, MacFarlane SM, Churchill TA, Doyle JS, Sydora BC, Fedorak RN.** 2009. Oral administration of curcumin emulsified in carboxymethyl cellulose has a potent anti-inflammatory effect in the IL-10 gene-deficient mouse model of IBD. Dig Dis Sci **55:**1272–1277. https://doi.org/10.1007/s10620-009-0843-z.
22. **Vareed SK, Kakarala M, Ruffin MT, Crowell JA, Normolle DP, Djuric Z, Brenner DE.** 2008. Pharmacokinetics of curcumin conjugate metabolites in healthy human subjects. Cancer Epidemiol Biomarkers Prev **17:**1411–1417. https://doi.org/10.1158/1055-9965.EPI-07-2693.
23. **Walker MK, Boberg JR, Walsh MT, Wolf V, Trujillo A, Duke MS, Palme R, Felton LA.** 2012. A less stressful alternative to oral gavage for pharmacological and toxicological studies in mice. Toxicol Appl Pharmacol **260:**65–69. https://doi.org/10.1016/j.taap.2012.01.025.
24. **Wang XS, Zhang ZR, Zhang MM, Sun MX, Wang WW, Xie CL.** 2017. Neuroprotective properties of curcumin in toxin-base animal models of Parkinson's disease: a systematic experiment literatures review. BMC Complement Altern Med **17:**412-422. https://doi.org/10.1186/s12906-017-1922-x.
25. **Wang Y, Rishi AK, Wu W, Polin L, Sharma S, Levi E, Albelda S, Pass HI, Wali A.** 2011. Curcumin suppresses growth of mesothelioma cells in vitro and in vivo, in part, by stimulating apoptosis. Mol Cell Biochem **357:**83–94. https://doi.org/10.1007/s11010-011-0878-2.