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Original Article

Concentrations of total curcuminoids in plasma, but not liver and kidney, are higher in 18- than in 3-months old mice

Alexa Kocher [a,*], Stephanie Hagl [b], Christina Schiborr [a], Gunter P. Eckert [b], Jan Frank [a]

[a] Institute of Biological Chemistry and Nutrition, University of Hohenheim, Garbenstr. 28, D-70599 Stuttgart, Germany
[b] Department of Pharmacology, Campus Riedberg, Biocenter, University of Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany

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ABSTRACT

Background: Curcuminoids (curcumin, demethoxycurcumin, bis-demethoxycurcumin) are lipophilic polyphenols thought to be effective in the prevention and treatment of neurodegenerative disorders, of which mitochondrial dysfunction is a prominent feature. In particular, older people may thus benefit from increasing their curcuminoid intake. However until now, it is not investigated if there exist age differences in the bioavailability of curcuminoids and therefore, it is unclear if curcumin doses have to be adjusted to age. Thus, we explored if the tissue concentrations and biological activities of curcuminoids are affected by age.
Methods: We investigated age-differences in the bioavailability and tissue distribution of curcuminoids and mitochondrial function in 3- and 18-months old mice fed a control diet or identical diets fortified with 500 or 2000 mg curcuminoids/kg for 3 weeks. Therefore, we measured curcuminoid concentrations in plasma, liver, kidney, and brain, basal and stress-induced levels of adenosine triphosphate (ATP) and mitochondrial membrane potential (MMP) in dissociated brain cells and citrate synthase activity of isolated mitochondria.
Results: Plasma but not liver and kidney curcuminoid concentrations were significantly higher in older mice. Age did not affect ATP concentrations and MMP in dissociated brain cells. After damaging cells with nitrosative stress, dissociated brain cells from old mice had a higher MMP than cells from young animals and were therefore more resistant. Furthermore, this effect was enhanced by curcumin.
Conclusion: Our data suggest that age may affect plasma concentrations, but not the tissue distribution of curcuminoids in mice, but has little impact on mitochondrial function in brain cells.

1. Introduction

Curcuminoids are lipophilic polyphenols derived from the rhizome of the plant *Curcuma longa* and responsible for the yellow color of turmeric. Commercially available curcumin powders contain 75–85% curcumin, 15–20% demethoxycurcumin (DMC) and 2–8% bis-demethoxycurcumin (BDMC) [1]. Curcuminoids are used as a spice and food additive (E100) [2] and have long been used in Ayurvedic medicine for the treatment of respiratory and liver disorders, anorexia, rheumatism, diabetic wounds, runny nose, cough and sinusitis [7]. Curcumin, the major curcuminoid, may be effective in the prevention [4] and treatment [13] of neurodegenerative disorders, of which mitochondrial dysfunction is a prominent feature. Curcuminoids improve mitochondrial function and increase mitochondrial membrane potential (MMP) and adenosine triphosphate (ATP) concentrations in the brain of mice [6].

In particular, older people may thus benefit from increasing their curcuminoid intake. The oral bioavailability of curcuminoids is low [22] and the small fraction of absorbed curcuminoids is quickly metabolized by phase I and II enzymes and rapidly excreted from the organism [19,24]. During phase I metabolism, curcuminoids are reduced to dihydro-, tetrahydro-, hexahydro-, and octahydrocurcminoids and during phase II metabolism the reduced metabolites are conjugated with glucuronic acid and sulphate in the liver and intestine [10,11]. Physiological changes during aging, such as decreases in i) the absorption surface in the gastro-intestinal tract, ii) hepatic blood flow and mass, iii) renal blood flow and glomerular filtration rate, and iv) increases in total body fat, as well as v) changes in the activity of metabolic enzymes and transporters may affect the bioavailability of curcuminoids. It is currently not known, however, if the absorption and tissue concentrations of curcuminoids and consequently their biological activities are affected by age.

We compared young (3-months) and old (18-months) mice fed curcuminoids for three weeks to investigate if age impacts on curcuminoid concentrations in tissues (plasma, liver, kidney, and brain) and alters mitochondrial function in brain cells.

* Corresponding author. Tel.: +49 711459 24265.
E-mail address: alexa.kocher@nutrition-research.de (A. Kocher).
URL: http://www.nutrition-research.de (A. Kocher).

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2. Materials and methods

2.1. Animals and treatment

Thirty 3-months and thirty 18-months old male C57BL/6 mice were purchased from Janvier Labs (Le Genest-Saint-Isle, France) and divided into six groups of ten mice. Young (Y) and old (O) mice were fed either a pelleted control diet (C1000, Altromin, Lage, Germany; control groups: YC and OC, respectively) or the control diet supplemented with 500 mg curcumin (Y500 and O500, respectively) or 2000 mg curcumin per kg diet (Y2000 and O2000, respectively) for 3 weeks. The curcuminoid extract used in the formulation of the diets was from Jupiter Leys (Cochin, Kerala State, India) and contained 82% curcumin, 16% demethoxycurcumin (DMC), and 2% bis-demethoxycurcumin (BDMC). The mice had free access to feed and water throughout the experiment and were housed in groups of 5 animals per cage in a conditioned room (temperature, 23 °C; relative humidity 55%; 12 h light/dark cycle). All experiments were carried out by individuals with appropriate training and experience according to the requirements of the Federation of European Laboratory Animal Science Associations and the European Communities Council Directive (Directive 2010/63/EU). Body weights were measured weekly and at the end of the trial. The mice were sacrificed by cervical dislocation and decapitation. Blood samples were centrifuged in heparinized vials (1308 ×*g*, 5 min, 4 °C) and plasma samples were stored at −80 °C. Liver and kidney were rinsed with sodium phosphate buffer, snap-frozen in liquid nitrogen and stored at −80 °C. The brain was quickly processed on ice after displacement of the cerebellum and brain stem.

2.2. Quantification of total curcuminoids

Sodium acetate buffer (0.1 M, pH 4–4.5) containing 1.6% EDTA (48.8 μM) and 2.5% ascorbic acid (25 μM) were added to 100 μL plasma. Tissue samples (~300 mg liver or kidney in 400 μL buffer; ~100 mg cerebellum in 200 μL buffer) were homogenized (Miccra D-8 homogenizer, ART Prozess- und Labortechnik GmbH & Co. KG, Müllheim, Germany) in the same buffer. Plasma and tissue samples were incubated with 1000 U β-glucuronidase (from Helix pomatia, Sigma, St. Louis, USA) dissolved in 0.1 M sodium acetate buffer (pH 4–4.5) for 45 min at 37 °C under agitation. After incubation, 1 mL extraction solvent (95% ethyl acetate, 5% methanol, Carl Roth GmbH + Co.KG, Karlsruhe, Germany) was added and vortex -mixed for 30 s. Subsequently, samples were centrifuged (10,500 ×*g*, 5 min, 4 °C) and supernatants collected. This step was repeated twice. The organic layer was evaporated to dryness using an RVC 2–25 CDplus centrifugal evaporator (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany).

Samples were re-suspended in 150 μL methanol, vortex-mixed for 30 s, left in the dark for 10 min, and vortex-mixed again for 20 s, and then transferred to an injection vial. Curcuminoids were quantified on a Jasco HPLC system (Jasco GmbH, Gross-Umstadt, Germany) with a fluorescence detector (excitation wavelength 426 nm, emission wavelength 536 nm) and separated on a Reprosil-Pur C18-AQ column (150 mm × 4 mm, 3 μm particle size; Dr. Maisch GmbH, Ammerbuch, Germany) maintained at 40 °C. The mobile phase consisted of 52% deionized water (adjusted to pH 3 with perchloric acid), 34% acetonitrile and 14% methanol and was delivered at a flow rate of 1.4 mL/min. Curcuminoids were quantified against external standard curves (curcumin, purity ≥97.2%, CAS # 458-37-7; demethoxycurcumin (DMC), purity ≥98.3%, CAS # 22608-11-13; bis-demethoxycurcumin (BDMC), purity ≥99.4%, CAS # 24939-16-0; Chromadex, Irvine, USA).

2.3. Preparation of dissociated brain cells

Dissociated brain cells (DBC) were prepared as previously described [8,9]. After preparation, DBC were diluted in Dulbecco's Modified Eagle Medium (DMEM) without supplements and seeded in 24-well plates (250 μL for measurement of mitochondrial membrane potential) or 96 well-plates (50 μL for measurement of adenosine triphosphate (ATP)) and cultured in a humidified incubator at 37 °C under 5% CO_2. To induce nitrosative damage, DBC were incubated for 3 h with sodium nitroprusside (SNP; 0.1 mmol/L for ATP measurements; 2 mmol/L for mitochondrial membrane potential measurements) in DMEM (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany). The degree of damage caused by SNP was calculated by normalization of the MMP and ATP values after SNP incubation to the corresponding basal values.

2.4. Determination of mitochondrial membrane potential

Mitochondrial membrane potential (MMP) was measured in cultivated DBC using the fluorescent dye Rhodamin 123 (R123) [6]. DBC were incubated in the dark for 15 min (37 °C, 5% CO_2) with 0.4 μmol/L R123 and then centrifuged (3000 rpm, 5 min) and washed with Hank's Balanced Salt Solution (HBSS) buffer (supplemented with Mg^{2+}, Ca^{2+}, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES); pH 7.4; 37 °C). After supplementing DBC with new HBSS, MMP was assessed by reading the R123 fluorescence at an excitation wave- length of 490 nm and an emission wavelength of 535 nm (Victor X3 2030 multilabel counter, Perkin Elmer, Rodgau-Jügesheim, Germany). Fluorescence values were normalized to cellular protein concentrations.

2.5. Quantification of adenosine triphosphate concentrations

The ViaLight® Plus bioluminescence kit (Lonza, Walkersville, USA) was used for the quantification of ATP in DBC. Briefly, DBC were incubated for 10 min with lysis buffer and at least for 5 min with monitoring reagent. The emitted light (bioluminescence), which is linearly related to ATP concentrations, was recorded using a luminometer (Victor X3). ATP concentrations were quantified with a standard curve and normalized to cellular protein content.

2.6. Isolation of brain mitochondria

Brain (1/4) from the frontal region was homogenized in 2 mL of mitochondrial respiration medium (MiR05, containing EGTA (0.5 mmol/L), magnesium dichloride (3 mmol/L), lactobionic acid (60 mmol/L), taurine (20 mmol/L), potassium dihydrogenphosphate (10 mmol/L), HEPES (20 mmol/L), sucrose (110 mmol/L) and essentially fatty acid-free bovine serum albumin (1 g/L)). Additionally, a protease inhibitor (Roche, Mannheim, Germany) was added to the medium. Samples were homogenized and centrifuged (1400 ×*g*, 7 min, 4 °C). The supernatant was collected and centrifuged again for washing (1400 ×*g*, 3 min, 4 °C). Afterwards, the supernatant was centrifuged to collect mitochondria in the pellet (10,000 ×*g*, 5 min, 4 °C). After resuspension, mitochondria were centrifuged for washing (1400 ×*g*, 3 min, 4 °C) and once again (10,000 ×*g*, 5 min, 4 °C) to collect the mitochondria in the pellet.

2.7. Citrate synthase activity

An aliquot of the isolated mitochondria was immediately frozen in liquid nitrogen for citrate synthase activity measurement. A reaction medium containing 0.1 mmol/L 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), 0.5 mmol/L oxaloacetate, 50 μmol/L EDTA, 0.31 mmol/L acetyl coenzyme A, 5 mmol/L triethanolamine hydrochloride, 0.25% Triton X-100 and 0.1 mol/L Tris–HCl was mixed and incubated for 5 min at 30 °C. Then, 10 μL of mitochondria was added to the reaction medium and citrate synthase activity was determined spectrophotometrically at 412 nm.