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Curcumin Micelles Improve Mitochondrial Function in a Mouse Model of Alzheimer's Disease

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Abstract

Preventive strategies for late-onset Alzheimer's disease (AD) should start early at a prodromal stage. Mitochondrial dysfunction has been found to play an important role in the initiation of both aging and the pathogenesis of Alzheimer's disease. Curcumin, a widely used spice and food-coloring agent, is a polyphenol derived from the rhizome of Curcuma longa. It is known to have anti-oxidant, anti-inflammatory, and anti-protein-aggregate activities which are usually considered beneficial for mitochondrial function. We assessed brain mitochondrial function and concentrations of soluble Aβ40 in a mouse model of AD (Thy1-APP751SL transgenic mice) after 3-week administration of curcumin micelles. Curcumin micelles are a newly developed formulation that account for increased curcumin bioavailability. Curcumin treatment had positive effects on mitochondrial membrane potential and respiratory control ratio. Additionally, it decreased levels of soluble Aβ40 in brains of Thy1-APPSL transgenic mice. Hence, curcumin micelles are a promising neutraceutical for the prevention of AD.

Key words: Curcumin, bioavailability, mitochondria, prevention, Alzheimer.

Introduction

Alzheimer's Disease (AD) is a slowly progressing neurodegenerative disease which, in its sporadic form, mostly affects elderly people. Since the most important risk factor for AD is age, AD prevalence increases with the rising life expectancy. The exact cause of AD development still remains unclear although various hypotheses exist, suggesting for example β-amyloid and hyperphosphorylated tau proteins as key players in disease formation (1). Recently, clinical trials using disease modifying drugs failed and initiation of the treatment at mild to moderate stages of the disease was blamed as being too late to be effective (1). Thus, disease prevention at an early stage comes into focus. Recently, mitochondrial dysfunction has emerged to play an early role in both aging and AD development (2). According to these findings, substances that stimulate mitochondrial function would be promising candidates for the prevention of AD.

Curcumin, a component of the Indian spice turmeric (curcuma longa), has been found to exhibit several health-promoting effects, amongst them anti-oxidative, antibacterial, anti-inflammatory, anti-protein-aggregate and analgesic properties (3). Various preclinical and clinical studies have concluded that curcumin has preventive and therapeutic effects in neurodegenerative diseases (4, 5). According to its properties, curcumin seems to be a very suitable compound for the prevention of neurodegeneration in aging and AD development. Recently, we have shown that curcumin prevents mitochondrial dysfunction in the brain of senescence-accelerated mice (6). Curcumin bioavailability is generally low, which complicates its application as preventive agent. Therefore various approaches such as the co-administration with secondary plant compounds or incorporation into liposomes, phospholipids or nanoparticles attempt to improve bioavailability (7). Schiborr and coworkers have recently published that human plasma bioavailability is 185-fold increased after administration of curcumin micelles in comparison to native curcumin (8). In this work, we fed similar curcumin micelles to an AD mouse model (Thy1-APPSL transgenic mice) and examined its effect on brain mitochondrial function.

Materials and Methods

Animals and Treatment

Male C57BJ/6-Thy1-APP751SL (Thy1-APPSL) mice were bred in the animal facility of the Pharmacological Institute, University of Frankfurt. Thy1-APPSL mice express the human form of APP containing both the Swedish (KM670/671NL) and the London (V717L) double mutation under the murine Thy1 promotor. This promotor leads to increased and selective expression of APP in neurons (9). At the age of 7 months, animals were separated into groups of seven mice and fed with a pelleted control diet (C1000, Altromin, Lage, Germany)

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or an equivalent diet containing curcumin micelles (500mg curcumin/kg diet) for 3 weeks. The liquid curcumin micelles (NovaSOL Curcumin®; AQUANOVA AG, Darmstadt, Germany) were composed of 7% curcumin powder (equivalent to 6% curcumin) and 93% Tween-80 (Kolb, Hedingen, Switzerland; all percentages refer to weight) (8) and added to the oil used in the preparation of the diet.

Mice had free access to drinking water and the respective diet thoughout the trial and were sacrificed by cervical dislocation and decapitation. Blood samples were centrifuged in heparinized vials at 3000 rpm and plasma samples were stored at -80°C. The brain was quickly dissected on ice after removal of the cerebellum and brain stem.

Measurement of mitochondrial membrane potential, ATP level and mitochondrial respiration

Preparation of dissociated brain cells as well as measurement of mitochondrial membrane potential and ATP concentrations were accomplished using one brain hemisphere according to a previously published method (10). Isolation of brain mitochondria, high resolution respirometry and determination of citrate synthase activity were performed as described earlier (10). For the isolation of mitochondria, half of a brain hemisphere was used.

Quantification of curcuminoids

Quantification of curcuminoids in blood plasma and brain tissue samples was accomplished according to a method published by Schiborr and coworkers (11).

Brain amyloid ß quantification

Brain contents of soluble Aβ40 were determined in brain homogenate from transgenic Thy1-APPSL mice using a human Aβ40 ELISA Kit (Invitrogen, Camarillo, USA). One eighth of a mouse brain was homogenized in PBS spiked with a protease inhibitor (complete tablets by Roche, Basel, Switzerland). The brain homogenate was applied onto the included 96-well-plate and the assay was performed according to the manufacturer's instructions.

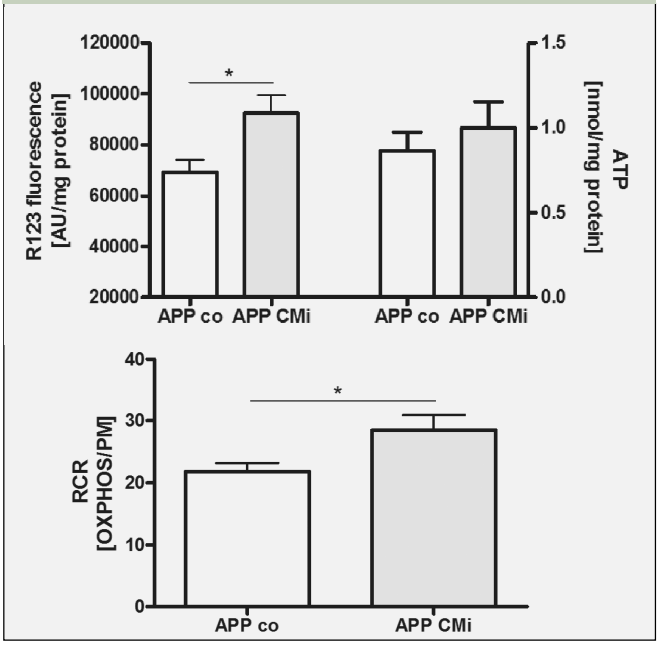
Statistics

Statistical analysis was performed by applying an unpaired Student's t-test (Prism 5.0, GraphPad Software, San Diego, CA, USA). A p-value of <0.05 was considered as statistically significant.

Results

Male Thy1-APP transgenic mice fed with pellets containing curcumin micelles significantly gained weight during the 3 week feeding period (mean weight difference between day 1 and day 22 of the trial: 2.4g) while the weight of mice fed with control pellets did not change (mean weight difference between day 1 and day 22 of the trial: 0g). The mean plasma concentrations of curcumin, demethoxycurcumin and bisdemethoxycurcumin were 543, 115 and 9nmol/L, respectively. In the brain, the concentrations of all three curcumin derivatives were below the limit of detection in all animals.

Figure 1. (A) basal mitochondrial membrane potential (R123 fluorescence, left) and basal ATP level (right) measured in dissociated brain cells and (B) respiratory control ratio (RCR) of isolated mitochondria from male Thy1-APPSL transgenic mice fed with a pelleted diet containing no additives (APP co) or curcumin micelles (APP CMi; 500 mg curcumin/kg diet) for 3 weeks; RCR was calculated as ratio between complex I + complex II respiration and leak respiration after addition of pyruvate and malate; n=7; mean±SEM; t-test; p*<0,05



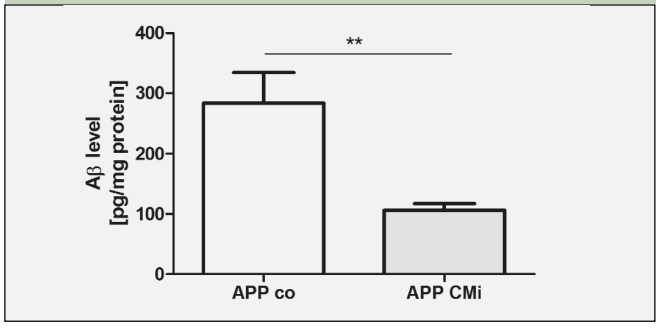
Curcumin administration significantly increased basal mitochondrial membrane potential (MMP) of dissociated brain cells (DBC) isolated from Thy1-APPSL transgenic mice (see Figure 1A). Basal ATP level were slightly but not significantly increased in DBC isolated from Thy1-APPSL transgenic mice (see Figure 1). In addition to basal ATP and MMP, we also determined ATP and MMP level in DBC after sodium nitroprusside (SNP) induced nitrosative stress. DBC were incubated with SNP for 3h (2mmol/L for MMP measurement; 0.5mmol/L for ATP measurement). After the incubation period, MMP had

dropped to 80% of control cell level while ATP dropped to 50% relative to control cells. Curcumin administration had no significant influence on the SNP induced decline in MMP and ATP concentrations (data not shown).

We determined mitochondrial respiration using High Resolution Respirometry (Oxygraph-2k, Oroboros, Innsbruck, Austria). By adding different substrates and inhibitors into the Oxygraph chambers, we were able to monitor respiratory states of complex I, complex II, complex I + complex II and complex IV as well as leak and uncoupled respiration (for detailed information see [10]). There were no significant differences in protein normalized respiration of isolated brain mitochondria between the two groups. The respiratory control ratio was calculated as the ratio between complex I + complex II respiration and leak respiration after addition of pyruvate and malate. Curcumin administration to Thy1-APPSL transgenic mice significantly increased RCR indicating improved coupling of the respiratory system (see figure 1B). We also determined citrate synthase (CS) activity, a marker that is highly correlated with mitochondrial content [12]. CS activity and respiration normalized to CS activity were not significantly different between the two groups.

Concentrations of soluble Aβ40 in brain homogenate from transgenic mice fed with curcumin diet were significantly decreased in comparison to brain Aβ levels determined in transgenic mice fed with control diet (see Figure 2).

Figure 2. Concentrations of soluble Aβ40 in brain homogenate from male Thy1-APPSL transgenic mice fed with a pelleted diet containing no additives (APP co) or curcumin micelles (APP CMi; 500mg curcumin/kg diet) for 3 weeks; n=7; mean±SEM; t-test; p**<0,01



Discussion

We found that a 3-week administration of curcumin micelles via a pelleted diet had an impact on brain mitochondrial function in Thy1-APPSL transgenic mice, a mouse model of Alzheimer's Disease. Curcumin feeding elevated mitochondrial membrane potential and increased respiratory control ratio in dissociated brain cells and isolated mitochondria from male Thy1-APPSL transgenic mice respectively. As discussed in the introduction, mitochondrial dysfunction has been suggested to be a key player in aging and the development of AD (2). According to these findings, curcumin, which improves mitochondrial function, might be a suitable agent for the prevention of neurodegeneration and AD.

Apart from increased Aβ concentrations and decreased performance in cognitive tests (13), mouse models of AD have been found to exhibit mitochondrial dysfunction. In Thy1-APPSL transgenic mice, MMP, ATP level and mitochondrial respiration were significantly decreased while reactive oxygen species production was significantly increased (14). Here, we showed that curcumin administration is able to ameliorate mitochondrial function in Thy1-APPSL transgenic mice by significantly increasing MMP and at least numerically increasing ATP level. By enhancing RCR, the coupling of the respiratory system is improved which in turn leads to increased MMP and improves functionality of the respiratory system (10).

In agreement with the current findings, we have recently reported that curcumin improves mitochondrial function in senescence-accelerated mice by influencing mitochondrial biogenesis and the fission/fusion balance (6). Another mechanism for the mitochondria-protective effects of curcumin is the reduction of oxidative stress either by direct interaction with oxidants or by up-regulating anti-oxidant enzymes (5). Furthermore, we could show that curcumin reduced levels of soluble Aβ40 in mouse brain of Thy1-APPSL transgenic mice. Similarly, curcumin has previously been reported to bind to amyloid plaques and reduce amyloid burden (5, 15), another mechanism probably contributing to its beneficial effects on mitochondrial function in Thy1-APPSL transgenic mice.

In spite of the vastly enhanced bioavailability of curcuminoids from micelles which was recently also demonstrated in humans (8), none of the three curcuminoids was detectable in mouse brain tissue at the end of the administration period. This is in agreement with our previous observations in mice administered 50 mg native curcumin per kg bodyweight by gastric intubation, in which no curcumin was detectable in the brain 30, 60 or 90 min after administration (11). At present, it is not known if the enhanced oral bioavailability of the micellar curcumin [8] may have led transiently to sufficiently high concentrations of curcumin to elicit biological activities in the brain. Thus, very small curcumin concentrations and/or curcumin metabolites may be responsible for the beneficial effects of dietary curcumin on mitochondrial function. Therefore a modulation of transcription factors, enzyme activities or gene expression seems to be more likely as a mechanism of action for curcumin than a direct antioxidative effect (5, 16).

In conclusion, daily consumption of micellar curcumin

for three weeks resulted in plasma concentrations of total curcuminoids in excess of 500 nmol/L, reduced Aβ burden in the brain and improved mitochondrial function in isolated mitochondria and dissociated brain cells in a mouse model of Alzheimer's disease. Micellar curcumin may thus be an interesting candidate for future human trials with people at risk of developing Alzheimer's disease.

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Conflicts of interest: Curcumin micells were provided by Aquanova in frame of a joint research project supported by the German Federal Ministry of Education and Reserach. The authors declare no conflicts of interest.

Ethical standards: All animal experiments were carried out by individuals with appropriate training and experience according to the requirements of the Federation of European Laboratory Animal Science Associations andt he European Communities Council Directive (Directive 2010/63/EU).

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