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Antidepressant-like effects of curcumin in WKY rat model of depression is associated with an increase in hippocampal BDNF

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Abstract

Curcumin is the principal active ingredient found in turmeric (*Curcuma longa*), a plant used in traditional Asian diets and herbal medicines. It is known to have a wide range of biological actions including antidepressant-like effects which have been observed in stress-induced depression models. This study was designed to investigate the antidepressant potential of curcumin in a non-induced model of depression. Moreover, since brain derived neurotrophic factor (BDNF) has been implicated in antidepressant effects of many drugs, we also evaluated the effects of curcumin on BDNF in the hippocampus. Adult male Wistar Kyoto (WKY) rats, a putative model of depression, were injected acutely or chronically (10 d) with 50, 100, and 200mg/kg curcumin. Open field locomotor activity (OFLA) and forced swim test (FST), a measure of helplessness, were measured 1 hour after acute and 18–20 hours after last chronic injection. Results showed a dose-dependent reduction of immobility in the FST by curcumin in both acute and chronic studies, without any significant effect on OFLA. The effect of higher chronic curcumin dose in FST was still evident a week later. Chronic curcumin also resulted in a dose-dependent increase in hippocampal BDNF. This data provides evidence for an antidepressant-like effect of curcumin, possibly through increased neurotrophic activity, in the WKY model of depression, and support the notion that curcumin may prove an effective and lasting natural antidepressant.

Depression is a severe disorder with many definitions and manifestations that has enormous consequences on an individual's quality of life, and it is among the most prevalent forms of mental illnesses. Symptoms vary, but often include anhedonia, disrupted sleep, lack of motivation, or emotional distress (e.g., anxiety). Recent discoveries now provide growing support for presence of cellular atrophy and neuronal loss in major depressive disorder (MDD), which can be reversed by the neurotrophic effects associated with antidepressants [1]. Converging evidence supports the underlying hypothesis that it is the neurotrophic effects of antidepressants that lead to their efficacy in treating MDD by increasing

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neurogenesis (see [1] for detailed review). For example, ample studies indicate that neurotrophins such as brain-derived neurotrophic factor (BDNF) may play a role in the pathophysiology of depression, and that antidepressants may in part exert their effects via regulation of BDNF [2]. Several clinical studies have even reported that serum BDNF levels are decreased in depressed patients, and that they can be normalized by antidepressant treatment [3]. However, while there are a number of clinically effective treatments that work via the serotonergic and noradrenergic neurotransmitter systems, a large segment of patients exhibit treatment-resistance (i.e., are non-responsive) to first-line interventions (e.g., selective serotonin reuptake inhibitors: SSRIs), or suffer from negative side effects [4]. This necessitates the development of new antidepressants with novel actions and fewer side effects, and one promising new drug is the natural product curcumin.

Traditionally curcumin, the active ingredient in turmeric (*Curcuma longa*), has been used as a common food additive and herbal medicine. Epidemiological studies have suggested that societies that widely use curcumin show reduced incidence of inflammation-influenced and cognitive function diseases [5, 6]. Empirical studies demonstrate that curcumin can also provide significant antidepressant effect in stress-induced behavioral studies [7–9], but effect on non-induced depression is not known. Our major hypothesis was that curcumin would impart a dose-dependent and long lasting antidepressant effect in Wistar Kyoto (WKY) rats, a putative non-induced animal model of depression, and that these behavioral effects would be reflected in an increase of BDNF expression in the hippocampus.

The WKY strain was first developed as a normotensive control for the spontaneously hypertensive rat strain [10], but was later found to demonstrate exaggerated immobility in the forced swim test (FST), a measure of helplessness or depressive-like behavior [11]. This behavior of WKY rats was shown to be associated with a decreased hippocampal volume [12]. Furthermore, the resistance of WKY to SSRIs has some researchers brand it as a non-responsive model [13] although it should be referred to as non-SSRI responsive.

In this study, 32 adult male WKY rats (14–15 weeks old) were obtained from Harlan Laboratories. Animals receiving the same treatment were pair housed through the duration of the experiment in a standard polypropylene shoebox cages (42 × 20.5 × 20 cm) on chip bedding. Animals were subjected to a 1-week acclimatization period upon their arrival, following which they were handled daily for 1 week to minimize any possible stress-related effects associated with exposure to a novel environment. Throughout the experiment, animals had free access to food (Harlan Tek Lab) and water. The room was maintained at 24–26 °C at 55–66% relative humidity, on a normal light cycle (7:00 AM–7:00 PM) throughout the duration of the study. All behavioral testing and injections occurred between 8:00 A.M. and 12:00 P.M. All experiments were carried out in accordance with NIH guidelines, as approved by the Institutional Animal Care and Use Committee of the Howard University.

Animals were divided into four groups (n=8/group), and received intraperitoneal (i.p.) injection of vehicle (0.5% sodium carboxy-methyl cellulose in saline), or 50, 100, or 200 mg/kg curcumin (Sigma-Aldrich, USA). Acute behavioral tests were conducted 1 hour after the injection. For chronic behavioral tests animals were injected once daily for 10 days and behavioral tests were carried out 18–20 h after the last injection. For the neurochemical study identical chronic injections were carried out but no behavioral testing was performed. In this case, 18–20 hours after the last injection animals were sacrificed by decapitation for brain collection.

Two behavioral tests were conducted. First, open field locomotor activity was measured for each animal during a 10 minute period. An open field activity monitoring cage (27 × 27 ×

20.3 cm, Med Associates, Inc., St. Albans, VT) was used to assess activity by measuring ambulatory counts [12]. Second, to assess helplessness, a modified FST was used to measure immobility of the rats [14]. Essentially, rats were individually placed into a cylinder filled with ~30 cm water ($25 \pm 1^\circ\text{C}$) to ensure that animals could not touch the bottom of the container with their hind paws or their tails. A time sampling scoring technique was used whereby the predominant behavior – immobility or swimming – in each 5-s period of the 300-s test was recorded blindly as previously described [12]. It is of relevance to note that WKY rats exhibit spontaneous immobility in the forced swim test; hence, there is no need to have a pretest exposure to forced swimming the day before as is customary in inducing helplessness in other strains [13,15].

For neurochemical studies, brains were rapidly removed, frozen on dry ice and stored at -80°C . Each frozen brain was later thawed on ice and hippocampus (bilateral) was dissected alternating between treatment groups as described previously [15]. Hippocampus was homogenized in lysis buffer (10 mM Tris-buffer, 5 mM EDTA, 150 mM NaCl, 0.5% Triton X-100 (v/v) with protease inhibitors (Sigma-Aldrich, St. Louis, MO). The protein concentration in each sample was determined using a BCA protein Assay Kit (Pierce Biotechnology Inc., IL), and equal protein amount (as confirmed by β -actin) was loaded in each immunoblot. The proteins were separated using the SDS-PAGE gel (12%) and transferred onto a nitrocellulose membrane. The membranes were blocked with a blocking reagent (5% nonfat milk in TBS buffer) for 30 minutes and incubated at 4°C overnight with the primary antibody BDNF (1:200, Santa Cruz Biotechnology Inc., Santa Cruz, CA). The membranes were washed with TBST (TBS buffer with 1% Tween-20) and blocked with the blocking reagent. Membranes were then incubated for 1 hour at room temperature in Goat Anti-Rabbit-HRP conjugated secondary antibody (1:3000 in TBS, Bio-rad Laboratories, CA). The membranes were then washed in the TBST washing solution and then visualized using enhanced chemiluminescent kits (Bio-rad Laboratories, CA). The intensity of the protein bands on the gel was quantified using ChemiDoc XRS system (Biorad Laboratories, CA).

All data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey HSD post hoc test when significant main effects were indicated. All analyses were two-tailed and $*p < 0.05$ was considered significant a priori.

Acute treatment with curcumin resulted in a dose-dependent decrease in FST immobility with the 200 mg/kg treatment group showing a significant decrease compared to the control [Figure 1: $F(3,28)=2.78$, $p < .043$]. Chronic treatment with curcumin also resulted in a dose-dependent decrease in FST immobility. However, in this case, both the 100 and 200 mg/kg treatment groups showed a significant decrease compared to control [Figure 1: $F(3,28)=9.93$, $p < .009$; $F(3,28)=9.93$, $p < .001$, respectively]. One week after the last chronic injection, the effect of 100 mg/kg was absent, but the effect of the 200 mg/kg was still evident [Fig 1: $F(2,21)=6.08$, $p < .001$]. This effect of 200 mg/kg was no longer evident after 2 weeks of rest [Fig 1: $F(2,21)=.881$, $p > .533$]. Open field locomotor activity was not altered by any treatment (data not shown), suggesting that the treatment effects of curcumin on FST were independent of any effects on general locomotion.

Western blot analysis showed that chronic curcumin treatment resulted in a dose-dependent increase in hippocampal BDNF level. Parallel to the behavioral effects, both 100 and 200 mg/kg doses resulted in significant increase in BDNF $F(3,28)=16.39$, $p < .001$; $F(3,28)=38.41$, $p < .001$, respectively, whereas the 50 mg/kg dose was without any significant effect (Fig 2).

The results of this study verify an antidepressant effect of curcumin in WKY rat model of depression. Thus, curcumin both acutely and chronically produced a dose-dependent antidepressant effect in a “non-inducible” rat model of depression. The effects of chronic treatment were observed up to one week after the cessation of treatment, indicating a relatively long lasting effect. Furthermore, chronic curcumin treatment resulted in increased BDNF expression in the hippocampus. Since BDNF has been directly implicated in cell survival and neurogenesis [2], and alterations in hippocampal neurogenesis have been linked to depression [1], it may be suggested that at least some of the antidepressant effects of curcumin may be mediated via enhanced neurogenesis in the hippocampus.

Whether the acute antidepressant effect of curcumin might have similar effects on hippocampal BDNF remains to be elucidated. However, it is unlikely that acute administration of curcumin could result in increase BDNF expression, as synthesis of new protein could take far more than an hour to materialize. Therefore, a likely mechanism of acute curcumin effect might entail interaction with down-stream signaling pathways and/or influencing other neurotransmitter/receptor molecules. The wide array of curcumin effects (e.g. anti-inflammatory, cognitive enhancement) suggests wide neurochemical effects of curcumin [6,16]. Indeed, curcumin interaction with various neurotransmitters implicated in the pathophysiology of depression, e.g., serotonin and dopamine [17], GABAergic system [18], and glutamatergic system [19] have been reported. In-vitro studies indicate a protective effect of curcumin against NMDA-mediated toxicity, suggesting inhibitory effects of curcumin on NMDA receptors [19]. This inhibition could contribute to the acute antidepressant effect, as we have found that ketamine, an NMDA receptor antagonist, has a rapid and lasting antidepressant effect in WKY rats [15]. The mechanism(s) involved in the acute effects of curcumin may also play a significant role in the chronic effects of curcumin. Hence, the multimodal mechanism of action of curcumin may offer a unique advantage to curcumin's pharmacotherapy in depression, especially since it lacks adverse effects. Beyond possible mono-therapy with curcumin, it is likely that combination of curcumin with other antidepressants could provide a better intervention in some depressed patients [20].

Overall, our results align with earlier studies on the antidepressant actions of curcumin in mice [8] and rats [7,9] where depression was induced by stress or olfactory bulbectomy. Chronic stress also down regulates hippocampal BDNF and the reduced ratio of phosphorylated cAMP response element-binding protein (pCREB) to CREB levels (pCREB/CREB), and is reversed by chronic curcumin [21]. Together, these studies provide strong support for hippocampal BDNF involvement in antidepressant effects of curcumin.

A major consideration in curcumin's actual applicability in treatment of depression is its bioavailability since the compound, when taken orally, is subject to low absorption. Furthermore, curcumin appears to undergo rapid metabolism leading to a short half-life, and limited tissue distribution [22]. Some of these challenges may be overcome by co-administration of compounds such as piperine which can improve not only the absorption of curcumin, but may also slow its breakdown, hence increasing its bioavailability [7,23]. Nanoparticle encapsulation based delivery of curcumin has also shown considerable promise by increasing the absorption rate, although it is unknown if this translates into an increase in tissue penetration [24]. Finally, use of curcumin analogs and bioconjugates, is another promising venue in increasing bioavailability and improving brain penetration [25].

In conclusion, the present studies support the notion that curcumin can be an effective and lasting antidepressant treatment. This effect is likely partially mediated through hippocampal BDNF changes, suggesting a possible involvement of neurogenesis.

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References

- [1]. Banasr M, Dwyer JM, Duman RS. Cell atrophy and loss in depression: reversal by antidepressant treatment. *Curr Opin Cell Biol.* 2011; 23(6):730–7. [PubMed: 21996102]
- [2]. First M, Gil-Ad I, Taler M, Tarasenko I, Novak N, Weizman A. The effects of fluoxetine treatment in a chronic mild stress rat model on depression-related behavior, brain neurotrophins and ERK expression. *J Mol Neurosci.* 2011; 45:246–55. [PubMed: 21479508]
- [3]. Gonul AS, Akdeniz F, Taneli F, Donat O, Eker C, Vahip S. Effect of treatment on serum brain-derived neurotrophic factor levels in depressed patients. *Eur Arch Psychiatry Clin Neurosci.* 2005; 255:381–6. [PubMed: 15809771]
- [4]. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry.* 2006; 163:1905–17. [PubMed: 17074942]
- [5]. Chandra V, Pandav R, Dodge HH, Johnston JM, Belle SH, DeKosky ST, et al. Incidence of Alzheimer's disease in a rural community in India: the Indo-US study. *Neurology.* 2001; 57:985–989. [PubMed: 11571321]
- [6]. Ng TP, Chiam PC, Lee T, Chua HC, Lim L, Kua EH. Curry consumption and cognitive function in the elderly. *Am J Epidemiol.* 2006; 164:898–906. [PubMed: 16870699]
- [7]. Bhutani MK, Bishnoi M, Kulkarni SK. Anti-depressant like effect of curcumin and its combination with piperine in unpredictable chronic stress-induced behavioral, biochemical and neurochemical changes. *Pharmacol Biochem Behav.* 2009; 92:39–43. [PubMed: 19000708]
- [8]. Kulkarni SK, Bhutani MK, Bishnoi M. Antidepressant activity of curcumin: involvement of serotonin and dopamine system. *Psychopharmacology (Berl).* 2008; 201:435–42. [PubMed: 18766332]
- [9]. Xu Y, Ku BS, Yao HY, Lin YH, Ma X, Zhang YH, et al. Antidepressant effects of curcumin in the forced swim test and olfactory bulbectomy models of depression in rats. *Pharmacol Biochem Behav.* 2005; 82:200–6. [PubMed: 16171853]
- [10]. Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. *Jpn Circ J.* 1963; 27:282–93. [PubMed: 13939773]
- [11]. Pare WP. Open field, learned helplessness, conditioned defensive burying, and forced-swim tests in WKY rats. *Physiol Behav.* 1994; 55:433–9. [PubMed: 8190758]
- [12]. Tizabi Y, Hauser SR, Tyler KY, Getachew B, Madani R, Sharma Y, et al. Effects of nicotine on depressive-like behavior and hippocampal volume of female WKY rats. *Prog Neuropsychopharmacol Biol Psychiatry.* 2010; 34:62–9. [PubMed: 19800382]
- [13]. Tejani-Butt S, Kluczynski J, Pare WP. Strain-dependent modification of behavior following antidepressant treatment. *Prog Neuropsychopharmacol Biol Psychiatry.* 2003; 27:7–14. [PubMed: 12551720]
- [14]. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature.* 1977; 266:730–2. [PubMed: 559941]
- [15]. Tizabi Y, Bhatti BH, Manaye KF, Das JR, Akinfiresoye L. Antidepressant-like effects of low ketamine dose is associated with increased hippocampal AMPA/NMDA receptor density ratio in female Wistar-Kyoto rats. *Neuroscience.* 2012; 213:72–80. [PubMed: 22521815]
- [16]. Sandur SK, Ichikawa H, Pandey MK, Kunnumakkara AB, Sung B, Sethi G, et al. Role of pro-oxidants and antioxidants in the anti-inflammatory and apoptotic effects of curcumin (diferuloylmethane). *Free Radic Biol Med.* 2007; 43:568–80. [PubMed: 17640567]
- [17]. Kulkarni SK, Akula KK, Deshpande J. Evaluation of antidepressant-like activity of novel water-soluble curcumin formulations and St. John's wort in behavioral paradigms of despair. *Pharmacology.* 2012; 89:83–90. [PubMed: 22343362]
- [18]. Gilhotra N, Dhingra D. GABAergic and nitriergic modulation by curcumin for its antianxiety-like activity in mice. *Brain Res.* 2010; 1352:167–75. [PubMed: 20633542]

- [19]. Matteucci A, Cammarota R, Paradisi S, Varano M, Balduzzi M, Leo L, et al. Curcumin protects against NMDA-induced toxicity: a possible role for NR2A subunit. *Invest Ophthalmol Vis Sci*. 2011; 52:1070–7. [PubMed: 20861489]
- [20]. Darvesh AS, Carroll RT, Bishayee A, Novotny NA, Geldenhuys WJ, Van der Schyf CJ. Curcumin and neurodegenerative diseases: a perspective. *Expert Opin Investig Drugs*. 2012; 21:1123–40.
- [21]. Xu Y, Ku B, Tie L, Yao H, Jiang W, Ma X, et al. Curcumin reverses the effects of chronic stress on behavior, the HPA axis, BDNF expression and phosphorylation of CREB. *Brain Res*. 2006; 1122:56–64. [PubMed: 17022948]
- [22]. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. *Mol Pharm*. 2007; 4:807–18. [PubMed: 17999464]
- [23]. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med*. 1998; 64:353–6. [PubMed: 9619120]
- [24]. Shaikh J, Ankola DD, Beniwal V, Singh D, Kumar MN. Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. *Eur J Pharm Sci*. 2009; 37:223–30. [PubMed: 19491009]
- [25]. Antony B, Merina B, Iyer VS, Judy N, Lennertz K, Joyal S. A Pilot Cross-Over Study to Evaluate Human Oral Bioavailability of BCM-95CG (Biocurcumin), A Novel Bioenhanced Preparation of Curcumin. *Indian J Pharm Sci*. 2008; 70:445–9. [PubMed: 20046768]

Highlights

- Curcumin exhibits antidepressant effect in WKY rat model of depression
- Antidepressant effect of curcumin is associated with an increase in hippocampal BDNF
- Curcumin may prove an effective and lasting antidepressant

Figure 1.

Effect of acute and chronic curcumin on FST immobility in male WKY rats. Acute immobility scores were obtained 1 h after the injection and chronic immobility scores were obtained 18–20 h after the last injection where rats were treated once daily for 10 days. Included are immobility scores at 1 and 2 weeks after the last injection. Bars represent SEM. Asterisks indicate significant difference from respective control, $p < 0.05$.

Figure 2.

Effect of curcumin on hippocampal BDNF in male WKY rats. Relative optical density of BDNF expression quantified via Western blot after 10 days of chronic curcumin injection. (A) Western blot sample beta-actin and BDNF, (B) Summary of all Westerns. n=8 per group. Bars represent SEM. Asterisks indicate significant difference from control, $p < 0.05$.