ORIGINAl ARTICLE

Restoration of Hypothalamic-Pituitary-Adrenal Axis Function in Rescue of Posttraumatic Stress Disorder in Curcumin-Treated Rats

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ABSTRACT

Curcumin, an active ingredient of Curcuma longa, has been reported to enhance serotonin levels in various regions of the brain. The aim of this study was to evaluate the effect of curcumin in the treatment of posttraumatic stress disorder (PTSD) and on cortisol levels. The effect of curcumin on PTSD was studied using the predator scent stress (PSS) model at two dose levels (5 and 10 mg/kg), two phases of treatment (short term, 14 days, and long term, 28 days) and in two categories of rats (unexposed and exposed to PSS). Behavioral parameters such as performance on an elevated plus maze (EPM) and freezing response on re-exposure to the PSS were observed. Serum cortisol levels were estimated using chemiluminescence and were compared with those in paroxetine-treated groups. Long term treatment with both dose levels of curcumin effected a marked increase in the number of open arm entries, while short term treatment had a significant effect only at 10 mg/kg. Cortisol levels were also increased significantly on long term treatment with curcumin. This decrease in the anxiety index and freezing response indicates the therapeutic potential of curcumin for treating PTSD. Further, the increased cortisol levels reinforce the restoration of HPA-axis function, which normally becomes dysfunctional in PTSD. Biomed. Int. 2011; 2: 72-80. ©2011 Biomedicine International, Inc.

**Key words:** Adrenal; behaviors; cortisol; curcumin; stress

INTRODUCTION

The incidence of anxiety disorders arising from current lifestyle changes is increasing rapidly and represents one of the major psychiatric problems driving patients to clinics. Among several types of anxiety disorders, PTSD is notable because of its distinctive symptomatology, which is different from traumatic stress and combat stress reactions and is developed after exposure to one or more terrifying events likely to cause psychological trauma. It is not age-related and is now acknowledged as a significant psychiatric illness in the civilian population. PTSD is characterized by recurrent, intrusive recollections of an overwhelming traumatic event experienced by the subject many years earlier. It is poorly recognized and diagnosed in clinical practice. Instead of considering it as a minor anxiety disorder, it needs to be recognized as a disorder in which memory consolidation and retrieval process are affected.

The neurobiology of PTSD, a stress-induced syndrome, can be addressed on the basis of the following hypothesis proposed by previous researchers: there are similarities with the affective disorder ‘depression’ in that hyperactivity of the hypothalamic pituitary adrenal axis (HPA) leads to increased corticotropin releasing factor (CRF) in both conditions, but...
cortisol levels are reported to rise in depression and to fall in PTSD. Altered catecholamine/sympathetic nervous system mechanisms are also reported to be involved in the etiology of PTSD, and disturbances in neurocognitive processing, especially sensory input and memory processing, are predominant in this condition. It can also be conceptualized as a disorder derived from disturbances of both episodic (declarative and conscious) and emotional memory with hypocortisolemia. The common symptoms of PTSD are re-experiencing, avoidance and hyperarousal; of these, ‘re-experiencing’ includes intrusive recollections of the trauma triggered by exposure to cues symbolizing that trauma, and is characterized by a dysfunctional anterior cingulate gyrus and intensification and impairment of memory. ‘Avoidance’ symptoms include diminished participation in activities and avoidance of thoughts or memories associated with the trauma. ‘Hyperarousal’ symptoms include difficulty in sleeping and concentrating, easy irritability, hypervigilance, and an increased startle response due to disturbances in sensory processing.

Curcumin, obtained from rhizomes of Curcuma longa, an indigenous medicinal herb, has been reported to be effective in many CNS disorders such as depression, epilepsy, Alzheimer’s, orofacial dyskinesia, etc. and to be neuroprotective. Curcumin has also been reported to affect the brain monoamine levels by inhibiting monoamine oxidase (MAO) and elevating levels of serotonin, a neurotransmitter deficient in cases of PTSD and widely involved in the biological response to traumatic stress. Serotonin reuptake inhibitors (SSRIs) are currently the only choice of drugs for treating PTSD and are associated with serotonin syndrome, constitutively characterized by changes in mental status, neuromuscular dysfunction and autonomic instability. Hence, an alternative drug for effective treatment of PTSD is needed, one that elevates serotonin levels and lacks the side effects of existing treatments. In the light of these reports, the present study was designed to elucidate the effect of curcumin on PTSD using exposure to predator scent-induced stress (PSS) as a model of PTSD with short term (14 day) and long term (28 day) treatments. After the traumatizing events, behavioral parameters such as freezing time and performance on an elevated plus maze (EPM) were assessed and the effect of curcumin on serum cortisol levels was observed.

**METHODOLOGY**

*Predator scent stress*

Exposure to a traumatic event (induction of PTSD) was achieved by placing the rats on well soiled cat litter (used by the cat for two days) for 10 min. The control animals were exposed to unused litter for the same period. Re-exposure to the cat litter on the last day of treatment before assessing EPM performance served as a situational reminder during which freezing time was measured.

*Animals*

Inbred adult male Wistar rats (200-250 g) were obtained from the animal house of Bapatla College of Pharmacy (1032/ac/07/CPCSEA), Bapatla, India, and were housed at constant room temperature (22 ± 1°C) and 40-50% relative humidity with a 12 h/12 h light/dark cycle. Standard food pellets (Rayan’s Biotech, Hyderabad, India) and water were provided *ad libitum* throughout the experimentation period. Animals were acclimated to the laboratory conditions for one week prior to the initiation of the experiments. The experimental protocol was approved by the institutional animal ethics committee
and all of the experiments involved in this work were performed in accordance with CPCSEA guidelines for the care and use of experimental animals.

**Drugs and drug administration**

Curcumin was procured from CHEMILOIDS, Laila Impex, Vijayawada and characterized by H+ NMR studies. For oral administration, curcumin was mixed in peanut oil and diluted to the desired concentration with the same solvent on the day of administration. Paroxetine, 1.8 mg/kg p.o., was suspended in 1% carboxymethyl cellulose (CMC). The peanut oil and CMC were used as control treatments and the behavioral data did not differ between rats that received these vehicles, so the results were compared with the peanut oil (vehicle)-treated group (control).

**Experimental procedure**

Rats were randomly distributed to 16 groups (n=6). Groups I to VIII were used to study the effect of short term drug treatment and groups IX to XVI were used to study the effect of long term treatment with curcumin and paroxetine. Groups I to IV were exposed to unused litter and then treated respectively with peanut oil 0.1 ml/100g, curcumin 5mg/kg, curcumin 10 mg/kg p.o., and paroxetine 1.8 mg/kg p.o. in 1% CMC for 14 days. Groups V to VIII were exposed to unused litter and received the same treatments as Groups I-IV for 28 days. Groups IX to XII were exposed to predator scent (cat litter) for 10 min and treated as mentioned above, beginning 1 h after PSS exposure, for 14 days. Groups XIII to XVI were exposed to PSS for 10 min and treated in the same manner, beginning 1 h after PSS exposure, for 28 days. Drugs were administered and behavioral parameters such as freezing response and performance on EPM were estimated between 10.00 a.m. and 12.00 a.m. Serum cortisol levels were measured using a Siemens Automated Chemiluminescence system ACS-180 SE.

**Freezing response (duration)**

Group IX to group XVI were re-exposed to cat litter (situational reminder) 1 h after the final dose of treatment. The time of immobility (complete absence of movements) was observed over 10 min, and the rats were used for behavioral measurements in the EPM. No freezing response was expected in groups I to VIII as they were exposed to fresh litter.

**Elevated plus maze**

Behavioral responses in the EPM were assessed after the situational reminder and the number of entries into open arms and closed arms, the length of time spent in open and closed arms, and the total exploration (entries into all arms), were observed over 5 min. The anxiety index was calculated as follows.

$$\text{Anxiety index} = 1 - \left[ \frac{\text{time spent in the open arms}}{\text{total time on the maze}} + \frac{\text{number of entries to the open arms}}{\text{total exploration on the maze}} \right]_2$$

**Statistical analysis**

The data obtained from the performances on EPM, freezing responses and cortisol levels were expressed as means ± SEM and the results of each group were compared with unexposed and negative control (exposed) rats. All the data were statistically analyzed using one way ANOVA. When the F test ratio was significant, the inter-group differences were evaluated using Dunnet’s ‘t’ test and P< 0.05 was considered to be significant.
RESULTS

Effect of short term treatment with curcumin on EPM performance

Curcumin (10 mg/kg) was associated with a marked increase (p<0.01) in the number of open arm entries by unexposed rats, whereas 5 mg/kg curcumin only caused a significant (p<0.01) increase in open arm entries by rats exposed to PSS. A perceptible decrease in open arm entries was observed in the negative control rats (Group IX) after exposure to PSS. A significant increase in the number of open arm entries by unexposed (p<0.01) and exposed (p<0.001) rats was observed after paroxetine treatment (Figure 1).

![Fig. 1. Effect of short term treatment of curcumin in rats unexposed and exposed to PSS](image)

Figure 1: Shows the effects of short term treatment with curcumin and paroxetine on rats unexposed and exposed to PSS. The graph represents the means ± SEM (n=6) of the number of open arm entries. The values for 5 and 10 mg/kg curcumin and paroxetine were compared with control (Group I): F = 5.02, d.f. = 3, 20 in unexposed rats; and with negative control (Group IX): F = 3.5 d.f. = 4, 25 in exposed rats. *p<0.05; **p<0.01; #p<0.001; ns = not significant.

Effect of long term treatment with curcumin on EPM performance

The number of open arm entries by groups VI and VII (unexposed to PSS and treated with 5mg/kg and 10 mg/kg curcumin, respectively) was significantly greater than control (Group V). The number of open arm entries by groups XIV and XV (exposed to PSS and treated with 5mg/kg and 10 mg/kg, respectively) was significantly greater than the negative control (Group XIII). A significant increase in the number of open arm entries was observed with paroxetine treatment in both unexposed (p<0.01) and exposed (p<0.001) rats (Figure 2).

Effect of short term and long term treatment with curcumin on freezing response

After exposure to PSS, a significantly longer freezing time was observed in the negative control groups, IX and XIII, than in the control groups, i.e. groups I and V respectively. Short term treatment with 5 and 10 mg/kg curcumin led to significant decreases in freezing time (p<0.05 and p<0.01 respectively). On long term treatment with 5 and 10 mg/kg curcumin, significantly shorter freezing times (p<0.01 and p<0.001) were observed in rats exposed to PSS than in the negative control groups. Treatment with paroxetine caused a significant decrease (p<0.001) in freezing time in both groups XII and XVI (short term and long term treatments) (Figure 3).
Fig. 2. Effect of long term treatment of curcumin in rats unexposed and exposed to PSS

![Graph showing the effect of long term treatment of curcumin in rats unexposed and exposed to PSS.](image)

Figure 2: Shows the effects of long term treatment (28 days) with curcumin and paroxetine on rats unexposed and exposed to PSS. The graph represents the mean ± SEM (n=6) of the number of open arm entries. The values for 5 and 10 mg/kg curcumin and paroxetine were compared with control (Group V): F = 5.23 d.f. = 3, 20 in unexposed rats; and with negative control (Group XIII): F = 4.96 d.f. = 4, 25 in exposed rats. *p<0.05; **p<0.01; #p<0.001; ns = not significant.

Fig. 3. Effect on freezing time in rats exposed to PSS

![Graph showing the effect on freezing time in rats exposed to PSS.](image)

Figure 3: Shows the effect of curcumin and paroxetine on freezing time after A. short term and B. long term treatment of rats exposed to PSS. The graph represents means ± SEM (n=6). The values of control, 5 and 10 mg/kg curcumin and paroxetine were compared with negative control (Group IX, XIII): F= 6.702 (short term), F= 8.33 (long term) d.f. = 4, 25. *p<0.05; **p<0.01; #p<0.001; ns = not significant.

Effect on cortisol levels

Short term administration of unexposed rats with curcumin caused no significant change in cortisol levels, but a significant (p<0.05) increase in cortisol levels was observed after long term treatment with 10 mg/kg curcumin (group VII). Paroxetine treatment caused no significant increase in unexposed groups, i.e. groups IV and VIII (Figure 4). However, rats exposed to PSS showed a significant increase in cortisol levels (p<0.01) on short term administration with 10 mg/kg curcumin (group XI), and significant increases in cortisol levels (p<0.05 and p<0.001 respectively) were observed after long term administration of 5 and 10 mg/kg curcumin (groups XIV and XV). Paroxetine on short term administration
caused no significant change, but long term administration effected a significant increase (p<0.05) in cortisol levels (Figure 5).

**DISCUSSION**

This study demonstrated that exposure to PSS induced symptoms of PTSD in rats. The increase in freezing response time in rats re-exposed to the PSS resembles the re-experiencing symptoms of PTSD in humans, confirming the face validity of the animal model (similarity in symptoms to the human condition). The increase in the number of open arm entries by curcumin and paroxetine treated rats shows the predictive validity of the model (similarity in response to treatment). The increase in cortisol levels in the curcumin treated groups shows its construct validity (similarity in underlying mechanisms of disorder).

Various symptoms of PTSD, re-experiencing, avoidance of stimuli and hyperarousal, are associated with the traumatic event. In general, an automatic response comes from the amygdala to the HPA axis, sympathetic and parasympathetic systems, with stress as an immediate reaction. This leads to elevation of corticotropin releasing factor (CRF), which stimulates cortisol release from the adrenal gland. Cortisol weakens the aversive memory trace and reduces the symptoms of stress, but the cellular mechanisms of PTSD include dysregulation of the HPA axis: there is hypersecretion of CRF but subnormal levels of cortisol. The importance of cortisol is that it interrupts the cycle of retrieving, re-experiencing and reconsolidation of traumatic memories, and promotes forgetting or weakening of the aversive memory trace. The absence or deficiency of cortisol in PTSD does not interrupt the retrieving or re-experiencing of traumatic events, as each re-experience causes consolidation of the aversive memory trace, further sustaining the disorder.
Serotonin levels are markedly reduced in stress disorders, and accordingly the function of the HPA axis is altered in PTSD patients. Although antidepressants such as SSRIs exhibit their therapeutic effect by elevating serotonin levels, they do not influence the HPA axis. Moreover, they are associated with many side effects, so drugs that elevate both serotonin and cortisol levels and lack side effects could be of great therapeutic value in the treatment of PTSD. In our previous studies we found that curcumin elevates the serotonin levels in cortical, hippocampal and medullary regions of the rat brain. Serotonin receptors are located exclusively within the CNS and are most abundant in regions associated with learning and memory processes, including the cortex, hippocampus and striatum, implicating serotonin in regulating memory. Serotonin levels are reported to be decreased in PTSD. The increase in cortisol levels and decrease in the anxiety index of curcumin treated groups could be consistent with a weakening of aversive memory recollection and reconsolidation by cortisol. This could be substantiated by studies showing that cortisol administration significantly reduces traumatic memories after exposure to a traumatic event. Thus, the curcumin-induced elevation of serum cortisol levels observed in the present study and of serotonin levels observed in our previous study, and the reported inhibition of MAO by curcumin, could have contributed towards its protective effect against PSS-induced PTSD.

CONCLUSION

SSRIs are at present the most widely used drugs for treating PTSD. They are reported to restore the altered serotonergic activity without affecting the HPA axis. In light of the above findings it appears that curcumin could be a superior drug of choice for treating PTSD, as it elevates both serotonin and cortisol levels and protects patients from the untoward effects of the existing drugs, ensuring safe treatment.
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REFERENCES