Effect of Curcumin on Diabetic Neuropathy in Streptozotocin Induced Diabetes in Rats

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Abstract: The mechanisms involved in diabetic neuropathic pain are complex and involve peripheral and central pathophysiological phenomena. Proinflammatory tumour necrosis factor α (TNF-α) and TNF-α receptor 1, which are markers of inflammation, contribute to neuropathic pain. The purpose of this experimental study was to evaluate the effect of curcumin on diabetic pain in rats. 24 rats were tested with diabetes induced by a single intraperitoneal injection of streptozotocin and 24 healthy control rats. Twelve rats in each group received 60 mg/kg oral curcumin daily for 28 days, and the other 12 received vehicle. On days 7, 14, 21, and 28, we tested mechanical allodynia with von Frey hairs and thermal hyperalgesia with radiant heat. Markers of inflammation in the spinal cord dorsal horn on day 28 were estimated with a commercial assay and Western blot analysis. Compared to control rats, diabetic rats exhibited increased mean plasma glucose concentration, decreased mean body weight, and significant pain hypersensitivity, as evidenced by decreased paw withdrawal threshold to von Frey hairs and decreased paw withdrawal latency to heat. **In conclusions:** Curcumin significantly attenuated the diabetes-induced allodynia and hyperalgesia and reduced the expression of both TNF-α and TNF-α receptor 1. Curcumin seems to relieve diabetic hyperalgesia, possibly through an inhibitory action on TNF-α and TNF-α receptor 1.
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1. Introduction

Diabetes mellitus is a chronic metabolic disorder related to insulin deficiency and can involve many organs. Diabetic neuropathy is a common complication that affects sensory neurons, motor neurons, and the autonomic nervous system. Diabetic pain is one of the most common symptoms of diabetic neuropathy (NF-κB), and mitochondrial dysfunction [2,3,5].

Various studies have shown that glial cells, particularly microglia, are activated in uncontrolled hyperglycaemic conditions in the spinal cord [8,9,10]. After undergoing phenotypic changes, activated microglia release various proinflammatory cytokines, including interleukin 1β (IL-1β) and tumour necrosis factor α (TNF-α), which have been implicated directly in the induction of neuropathic pain [9]. Although the role of microglia and their pharmacological modulation are poorly understood, the expression of proinflamatory cytokines in the dorsal horn of the spinal cord is thought to contribute to the pathogenesis of diabetic neuropathy.

Curcumin is a naturally occurring polyphenolic pigment extracted from the Curcuma longa, which has antitumor, antioxidant, and antiinflammatory properties [11]. What's more, curcumin is well absorbed and has good tissue penetration, including through the blood brain barrier [12]. The mechanism that underlies diabetic pain is complicated. The pathogenesis of hyperglycaemia related diabetic neuropathy involves multiple mechanisms, including oxidative and nitrosative stress, hyperalgesia, activation of nuclear factor-κB glucose analyser[1]. Rats with fasting plasma glucose levels higher than 300 mg/dL were deemed to be diabetic and were included in the study. Body weight and plasma glucose levels were recorded twice weekly for the duration of the study. The aim of the present study was to evaluate the anti-inflammatory effect of curcuminon diabetic neuropathy in streptozotocin induced diabetes in rats.

2. Materials and methods

Animals

Eighty four male albino rats weighing (200-250 gm) were used. They were housed at ordinary room temperature, were maintained on a 12 hour light-dark cycle, and had free access to food and water.

Induction of diabetes in 24 rats was by administering one dose of streptozotocin prepared in citrate buffer. The streptozotocin was injected intraperitoneally at a dose of 65 mg/kg. Twenty four age-matched control rats were administered an equal volume of citrate buffer vehicle. Plasma glucose levels were estimated with a commercial blood glucose analyser (Accusoft, Roche Diagnostics, Laval, QC,

Canada). Body weight and plasma glucose levels were recorded twice weekly for the duration of the study.

Diabetic and control rats were randomly selected and divided into four groups of 12 animals each. Twelve diabetic rats and 12control rats received oral curcumin treatment (60 mg/kg) daily from day3 to day 28. The remaining 12 diabetic rats and 12 control rats were treated with curcumin vehicle (0.5% sodium carboxy-methylcellulose) over the same time period. Drug and vehicle were prepared immediately before administration and were delivered in a constant volume of 5 mL/kg body weight.

Assessment of mechanical allodynia

Paw withdrawal thresholds were measured 1 day before injection of streptozotocin or vehicle and on days 7, 14, 21, and28 after injections. Rats were placedin cages with mesh floors and covered with transparent plastic boxes. They were allowed to acclimatize to their surroundings for a minimum of 20 min in a temperature controlled room (25ºC) before being tested. Von Frey hairs in log increments of force (0.38, 0.57, 1.23,1.83, 3.66, 5.93, 9.13, and 13.1 g) were applied for a duration of 4–6 s to the region between the foot pads in the plantar aspect of the hind paw. Abrupt paw withdrawal, licking, and shaking were taken to be positive responses.

Assessment of thermal hyperalgesia

To assess nociceptive responses to thermal stimuli, we placed rats in animal holders with gentle restraint and applied radiant heat to the plantar surface of the test paw. The thermal withdrawal latency from the radiant heat was recorded with a plantar test (Hargreaves' method) [19].Abrupt paw withdrawal, licking, and shaking were taken to be positive responses.

Estimation of TNF-α level

Rats were sacrificed with an overdose of inhalational isofluraneon day 28. The spinalcords were separated quickly and stored at −70ºC until they were ready to be processed for biochemical estimations. The spinal cord lumbosacral enlargement (L5) was homogenized in lysis buffer that contained 50 mMtris(hydroxymethyl)aminomethane (pH 8.0), 150 mM sodium chloride, and 1% 4-nonylphenyl-polyethylene glycol (Nonidet P-40, Sigma-Aldrich, USA). The homogenate was centrifuged at 5000 x g for 15 min at4ºC for isolation of total supernatant protein. Protein concentration was determined with Pierce BCA Protein Assay Kit (Thermo Scientific Pierce Protein Biology Products, Rockford, IL, USA) according to the instructions of the manufacturer. TNF-α concentration was measured with a rat TNF-α kit (R&D Systems, Minneapolis, MN, USA).

Expression of TNF-α receptor 1

We used Western blot analysis to measure expression of TNF-α receptor 1 in the spinal cord dorsal horn samples. The protein extracts were separated on7.5% polyacrylamide gels by electrophoresis and transferred on to polyvinylidene difluoride membrane.

Statistical analyses

All data are presented as mean and standard error of the mean (SEM). Differences between and within groups were compared with one-way analysis of variance (ANOVA). We also used pair-wise comparisons between groups, performed with the Student-test. Statistical significance was set at $p<0.05$. All analyses were carried out with SPSS version 13.0 (SPSS Inc., New York, NY, USA).

3. Results

Body weight and blood glucose levels

At 28 days after streptozotocin injection, diabetic rats exhibited significantly increased blood glucose levels and reduced body weight compared with control rats. Hyperglycaemia and bodyweight improved significantly in diabetic rats that were treated with curcumin compared with diabetic rats that were treated with vehicle (Table 1).

Table 1. Effect of curcumin on mean body weight and blood glucose levels.

Parameters Groups	Body weight (g)		Blood glucose (mg/dL)	
	Day 0	Day 28	Day 0	Day 28
Control + Vehicle	220±1.31	293±2.18	116±3.81	121±2.73
Control+ Curcumin	238±3.02	285±3.63	102±3.85	108±3.24
DM + Vehicle	240±1.57	195±2.42*	114±4.87	573±8.86**
DM + Curcumin	235±2.06	249±2.75	109±5.28	348±6.47#

* p = 0.02, ** p = 0.004 vs. Controls; # p = 0.01 vs. DM + Vehicle

Mechanical allodynia and thermal hyperalgesia

By day 28, paw withdrawal thresholds to von Frey hair stimulation were significantly lower in diabetic rats than in control rats, indicating the presence of mechanical allodynia. However, allodynia was less in diabetic rats treated with curcumin than in those treated with vehicle. Paw withdrawal thresholds did not differ significantly between the control groups during the entire observation period, irrespective of curcumin treatment (Fig.1A).

The threshold for thermal hyperalgesia was significantly decreased by day 14 after streptozotocin

injection compared to that of control rats and continued to develop up today 28indiabeticrats. Hyperalgesia was notably less in diabetic rats treated with curcumin than in diabetic rats treated with vehicle. (Fig.1B).

TNF-α and TNF-α receptor 1

TNF-α level in spinal cord dorsal horn was markedly higher in diabetic rats than in controls (274.36±18.97 pg/mg vs. 91.87±6.83 pg/mg). Diabetic rats that received curcumin had significantly lower levels of TNF-α than did those that received vehicle (158.33±12.18 pg/mg vs. 274.36±18.97 pg/mg). Curcumin treatment had no effect on spinal cord TNF-α level in control rats (92.14±6.59 pg/mg vs. 91.87±6.83 pg/mg; Fig.2).

The concentration of TNF-α receptor1inspinal cord dorsal horn was increased in diabetic rats compared with that in control rats. Treatment with curcumin reduced expression of TNF-α receptor 1 in diabetic rats compared to expression in rats that received vehicle (Fig.3). Curcumin had no effect on expression of TNF-α receptor1incontrol rats.

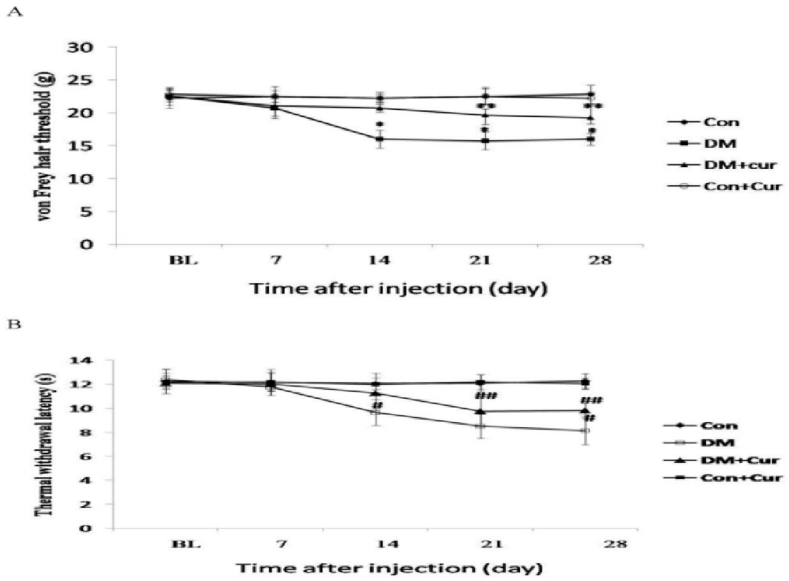

Figure 1. Effect of curcumin (Cur) treatment on the pain threshold in diabetic rats. Diabetes mellitus (DM) was associated with decreases in mechanical paw withdrawal threshold (A) and thermal paw withdrawal latency (B), but the decreases were significantly less in the curcumin-treated diabetic group than in the vehicle-treated diabetic group. Data are expressed as mean± S.E.M. (n=12 per group). BL, baseline; Con, control, *p=0.01 vs. control group; **p=0.03 vs. DM group; # p=0.01 vs. control group; ##p=0.04 vs. D M group.

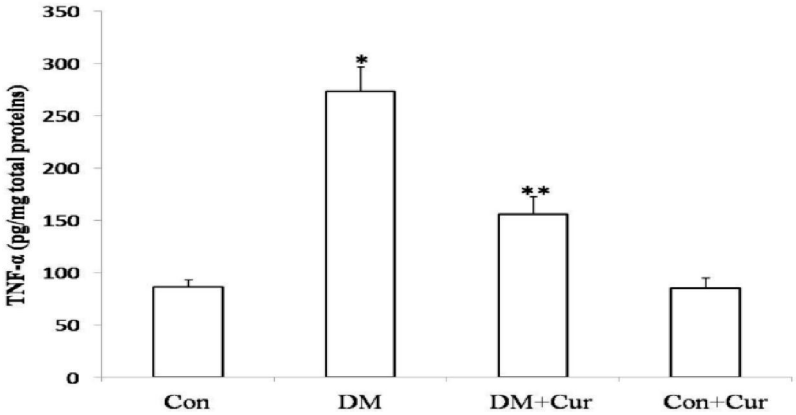

Figure 2. Mean± SEMTNF-α concentrations in spinal cord dorsal horn. Analyses were carried out with enzyme-linked immunosorbent assay; n=6 rats for TNF-α ELISA. *p=0.02 vs. control groups; Con, control, DM, diabetes mellitus; Cur, curcumin; **p=0.006 vs. DM group

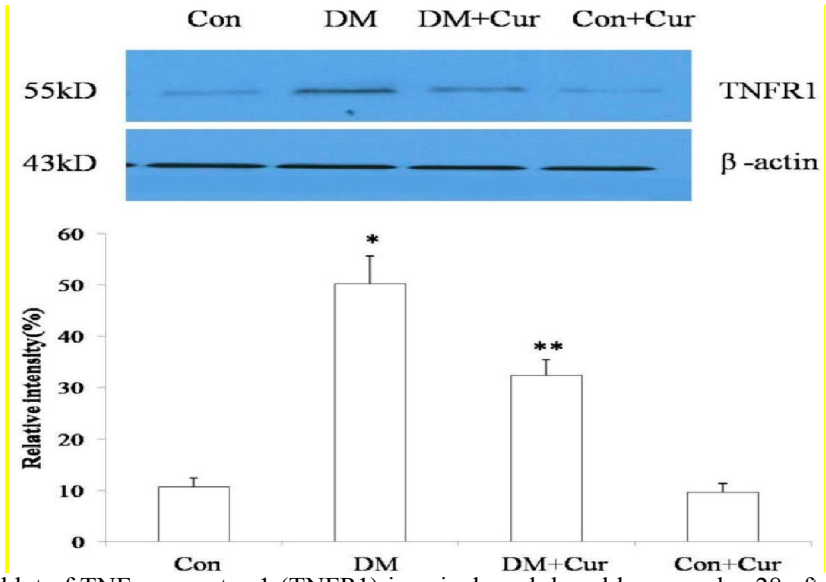

Figure 3. Western blot of TNF-α receptor 1 (TNFR1) in spinal cord dorsal horn on day 28 after rats were injected with strep- to zotocin (DM) or citrate buffer (Control [Con]). Results are mean±SE M of three independent experiments. Immunoblotting of β-actin confirmed equal loading. *p=0.01 vs. control group; **p=0.02 vs. DM group (n=6 per group).

4. Discussion

Evidence indicates that hyper glycaemia has toxic effects on neurons because of increased intracellular glucose oxidation, which leads to an increase in production of reactive species [13,14]. TNF-α concentration in spinal cord dorsal horn are elevated in several neuropathological disorders associated with hyperalgesia [15,16]. In the study, rats with streptocotozin-induced diabetes had significantly elevated blood glucose levels, decreased body weights, and reduced pain threshold compared with control rats. In diabetic rats treated with curcumin pain, the decrease in pain threshold was substantially less than that in diabetic rats treated with vehicle.

Previous preclinical studies have demonstrated an association between elevated TNF-α levels and altered pain behavior [7,17]. In the family of proinflammatory cytokines, TNF-α is regarded as a trigger for acytokine signaling cascade [18]. Interaction between TNF-α and TNF-α receptor1leads to the activation of NF-κB, which in turn induces transcription of genes that encode inflammatory and other pain related mediators, such as TNF-α, interleukin 6, and cyclooxygenase 2 [6,19].

It is well known that TNF-α generates pathological pain via peripheral actions. Increasing evidence suggests that TNF-α has central actions in pain sensitization. The levels of TNF-α and TNF-α receptor 1in dorsal root ganglia and spinal cord dorsal horn in- crease after peripheral nerve injury and in other neuropathic pain models. Intrathecal delivery of recombinant TNF-α (rTNF-α) can induce mechanical allodynia and thermal hyperalgesia in rats and intrathecal administration of the TNF-α inhibitor etanercept can attenuate inflammatory pain. Furthermore, TNF-α combined with transient receptor potential subtype V1 (TRPV1) can powerfully modulate spinal synaptic plasticity [20].

Pretreatment with the NF-κB inhibitor pyrrolidinedithiocarbamate can relieve mechanical allodynia and down regulate expression of TNF-α and TNF-α receptor 1 [19]. Studies have revealed that curcumin can attenuate thermal hyperalgesia in a diabetic mouse model by inhibiting serum TNF-α [21, 22]. In the present study, we found that increased pain hypersensitivity behavior in diabetic rats coincided with an increased expression of TNF-α and TNF-α receptor 1.

Curcumin can inhibit NF-κB activity and expression of inflammatory cytokines [23]. The expression of TNF-α and TNF-α receptor1in- creased markedly in spinal cord dorsal horn of experimental diabetic rats, but treatment with curcumin reduced the expression of both compared with vehicle treatment.

Finally, the result of this study indicate that the expression of TNF-α and TNF-α receptor1in spinal cord dorsal horn increase sin a diabetic rat model. Treatment with curcumin seems to lessen mechanical allodynia and thermalhyperalgesia through down regulation of TNF-α and TNF-α receptor 1 expression.

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