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Curcumin attenuates thermal hyperalgesia in a diabetic mouse model of neuropathic pain

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Abstract

Diabetic neuropathic pain, an important microvascular complication in diabetes mellitus is recognised as one of the most difficult types of pain to treat. A lack of the understanding of its aetiology, inadequate relief, development of tolerance and potential toxicity of classical antinociceptives warrant the investigation of the newer agents to relieve this pain. The aim of the present study was to explore the antinociceptive effect of curcumin and its effect on tumour necrosis factor-α (TNF-α) and nitric oxide (NO) release in streptozotocin induced diabetic mice. Four weeks after a single intraperitoneal injection of streptozotocin (200 mg/kg), mice were tested in the tail immersion and hot-plate assays. Diabetic mice exhibited significant hyperalgesia along with increased plasma glucose and decreased body weights as compared with control mice. Chronic treatment with curcumin (15, 30 and 60 mg/kg body weight; p.o.) for 4 weeks starting from the 4th week of streptozotocin injection significantly attenuated thermal hyperalgesia and the hot-plate latencies. Curcumin also inhibited the TNF-α and NO release in a dose dependent manner. These results indicate an antinociceptive activity of curcumin possibly through its inhibitory action on NO and TNF-α release and point towards its potential to attenuate diabetic neuropathic pain.

Keywords: Diabetic neuropathic pain; Hyperalgesia; Curcumin; Nitric oxide; TNF-α; Streptozotocin

1. Introduction

Neuropathic pain is generally considered to be one of the most common complications of diabetes, affecting both types of diabetes equally (Clark and Lee, 1995; Guy et al., 1985). It is mostly characterised by pain that can occur spontaneously as a result of exposure to mild painful stimuli, i.e., hyperalgesia (Brown and Asbury, 1984). Although neuronal loss or alteration of neurotransmitters (Greene et al., 1987) have been reported to be responsible for the changed pain perception, the exact etiologic factors remains unexplored. Several new drugs such as nonsteroidal anti-inflammatory drugs, antidepressants, anticonvulsants and opioids are currently under investigation in the management of diabetic neuropathic pain (James et al., 1999), but the treatment with these drugs is limited because of their partial effectiveness and potential toxicity (Arner and Meyerson, 1998; Courteix et al., 1993).

Renewed interest has been observed in recent years on the multiple activities of natural flavonoids. Curcumin, a yellow pigment from *Curcuma longa*, is a major component of turmeric and exhibits anticarcinogenic and anti-inflammatory properties including an inhibitory effect on the production of interleukin-8 (IL-8), interleukin-1β (IL-1β) and TNF-α by lipopolysaccharide stimulated monocytes and alveolar macrophages (Chan, 1995). Curcumin most likely inhibits cell proliferation, cell-mediated cytotoxicity and cytokine production by inhibiting nuclear factor-kappaB (NF-κB) target genes involved in induction of these immune responses (Gao et al., 2004). Studies have revealed that increased concentration of TNF-α correlates well with increased concentrations of NF-κB as observed in Caco-2 cell lines (Mao et al., 2004). Low concentrations of curcumin have also been found to inhibit nitric oxide production, as measured by the amount of nitrite released into the culture medium (Johnston and DeMaster, 2003). Together

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with the ability of curcumin to inhibit NO and TNF-α release which cause nitrosative stress in diabetes and promote pain and inflammatory signals, respectively, the present study was designed to evaluate the effect of curcumin in diabetic neuropathic pain and an attempt was made to look for the participation of NO and TNF-α in curcumin's antinociceptive effect.

2. Materials and methods

2.1. Animals

Male albino mice of Laka strain (20–30 g) bred in Central Animal House, facilities of Panjab University were used in the present study. The animals were housed under optimal laboratory conditions, maintained on a natural light and dark cycle and had a free access to food and water ad libitum. Animals were acclimatized to laboratory conditions before the tests. All experiments were carried out between 0900 and 1700 h. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy guidelines for the use and care of animals.

2.2. Drugs and reagents

Streptozotocin and Curcumin used in the study were purchased from Sigma (St. Louis, MO, USA). Sulphanilamide, phosphoric acid and naphthylethylene diamine dihydrochloric acid was purchased from Loba Chemie (Bombay, India). A glucose oxidase peroxidase diagnostic enzyme kit was purchased from Span Diagnostic Chemicals, India. The TNF-α ELISA kits were purchased from Becton Dickinson, USA.

2.3. Induction and assessment of diabetes

A single dose of 200 mg/kg streptozotocin prepared in citrate buffer (pH 4.4, 0.1 M) was injected intraperitoneally to induce diabetes. The age-matched control mice received an equal volume of citrate buffer and used along with diabetic animals. Diabetes was confirmed after 48 h of streptozotocin injection, the blood samples were collected through tail vein and plasma glucose levels were estimated by enzymatic GOD-PAP (glucose oxidase peroxidase) diagnostic kit method. The mice having plasma glucose levels more then 13.9 mmol/l (Anjaneyulu and Ramarao, 2002) were selected and used for the present study. Body weight and plasma glucose levels were also measured before and at the end of the experiment to see the effect of curcumin on these parameters.

2.4. Treatment schedule

After a basal reading at 4th week of streptozotocin injection, control and diabetic mice were randomly selected and divided in five groups of 6–7 animals each. First group consists of control animals, second group is the diabetic control and third, fourth and fifth group consisted of diabetic animals treated with curcumin (15, 30, and 60 mg/kg/day) orally. Starting from 4th week till 8th week, the control and diabetic control groups received vehicle of curcumin and other diabetic groups received suspension of curcumin at doses described above. Curcumin suspension was prepared in 0.5% carboxy methylcellulose solution. Drug suspension was freshly prepared and administered in a constant volume of 1 ml/100 g body weight.

2.5. Assessment of thermal hyperalgesia

2.5.1. Tail-immersion (warm water) test

Tail of mice was immersed in a warm water bath (52.5 ± 0.5 °C) until tail withdrawal (flicking response) or signs of struggle were observed (cut-off 12 s). Shortening of the tail withdrawal time indicates hyperalgesia and is attributed to central mechanisms (Kannan et al., 2003; Ramabadran et al., 1989).

2.5.2. Hot-plate test

The hyperalgesic response on the hot-plate is considered to result from a combination of central and peripheral mechanisms (Kannan et al., 2003). In this test, animals were individually placed on a hot-plate (Eddy's Hot-Plate) with the temperature adjusted to 55 ± 1 °C. The latency to the first sign of paw licking or jump response to avoid the heat was taken as an index of the pain threshold; the cut-off time was 10 s in order to avoid damage to the paw.

2.6. Nitrite estimation

Whole brain nitrite levels were estimated using Greiss reagent which served as an indicator of nitric oxide production (Di Rosa et al., 1990). Briefly, animals were sacrificed under deep anaesthesia and brains were removed and cleaned with ice-cold saline and homogenate was prepared with phosphate buffer (pH 7.4). 1.0 ml of Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid 0.1% naphthylethylene diamine dihydrochloric acid in water) was added to 1 ml of post mitochondrial supernatant of whole brain homogenate and absorbance was measured at 546 nm. Nitrite concentration was calculated using a standard curve for sodium nitrite and nitrite levels were expressed as percentage of control.

2.7. Estimation of TNF-α

TNF-α was estimated using a standard ELISA method. The estimations of TNF-α from the serum were performed by a sandwich ELISA method according to the manufacturer's instructions (Becton Dickinson, USA).

2.8. Statistical analysis

The nociceptive threshold, i.e. the latency (s) to thermal noxious stimuli, was measured and expressed as mean ± S.E.M. The hyperalgesic response was analysed by analysis of variance (ANOVA) followed by Dunnett's *t*-test to assess the

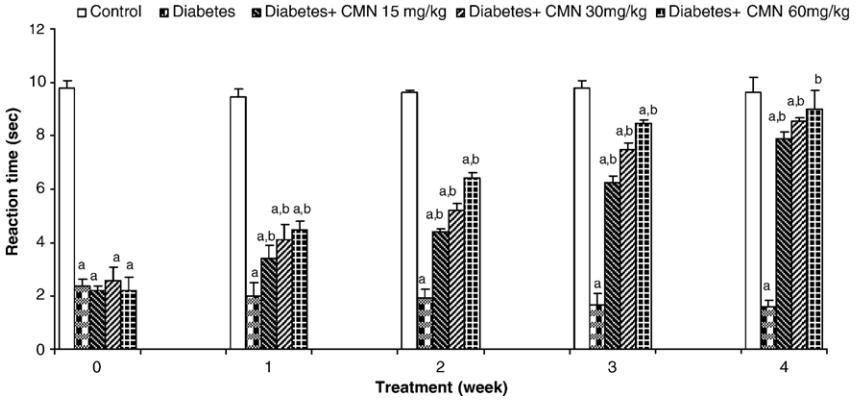

Fig. 1. Effect of curcumin (15, 30 and 60 mg/kg, p.o.) on the pain threshold values in streptozotocin-injected diabetic mice subjected to tail immersion in warm water (52.5±0.5 °C). Values are expressed as mean±S.E.M. ($n=7$ in each group). [a]$P<0.05$ as compared to control group. [b]$P<0.05$ as compared to streptozotocin (STZ)-treated diabetic group.

significance. $P<0.05$ was considered as statistically significant. Student's *t*-test was used to compare the values from two groups.

3. Results

3.1. Effect of streptozotocin-injection on blood glucose and body weights

Four weeks after streptozotocin injection, diabetic mice exhibited significantly increased plasma glucose levels (22.10±0.53 mmol/l) as compared to control mice (6.01±0.85 mmol/l; $P<0.05$). There was a marked decrease in the body weights of streptozotocin-injected mice (18.56±2.02 g) as compared with age matched control mice (29.42±1.46 g, $P<0.05$). Curcumin treatment (15, 30 and 60 mg/kg) from 4th to 8th week significantly decreased the plasma glucose levels to 20.23±0.12, 17.45±0.35 and 15.96±0.31 mmol/l, respectively, as compared to diabetic mice (25.83±1.25 mmol/l, $P<0.05$) at the end of the 8th week. The body weight was also significantly improved on treatment with curcumin in all three doses i.e. 21.56±0.56 g, 23.52±1.38 g and 25.96±1.19 g as compared to the diabetic mice (16.42±1.42 g, $P<0.05$), respectively.

3.2. Effect of chronic curcumin treatment on nociceptive threshold

At the end of the 4th week, diabetic animals exhibited decrease in pain threshold from noxious stimuli as compared to control rats ($P<0.05$). Curcumin administration (15, 30 and 60 mg/kg) for 4 weeks starting from 4th week significantly increased the pain threshold from 4th to 8th week compared to control diabetic mice ($P<0.05$, Figs. 1 and 2) in a dose-dependent manner, both in tail immersion and hot-plate assays.

3.3. Effect of curcumin on the serum TNF-α levels

Serum TNF-α levels were markedly increased in diabetic as compared to control mice. Curcumin (15, 30 and 60 mg/kg/day, p.o.) significantly decreased serum TNF-α levels as compared to the vehicle-treated diabetic mice ($P<0.05$, Fig. 3).

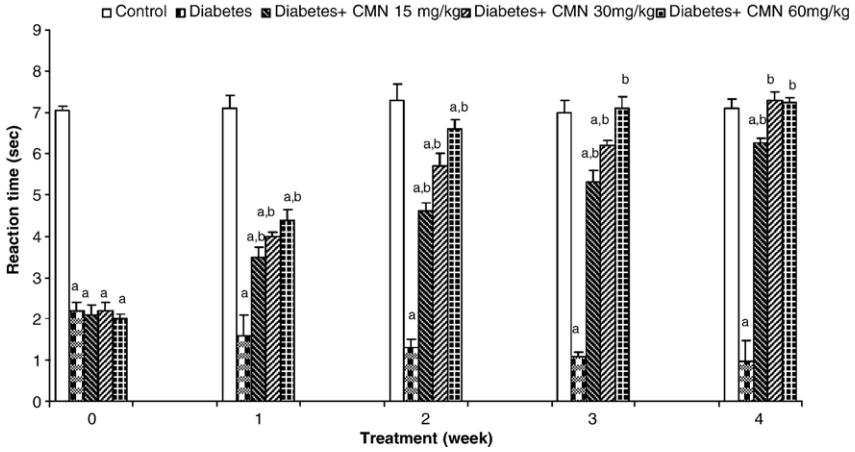

Fig. 2. Effect of curcumin (15, 30 and 60 mg/kg, p.o.) on the pain threshold values in streptozotocin-injected diabetic mice on the hot plate (55±1 °C). Values are expressed as mean±S.E.M. ($n=7$ in each group). [a]$P<0.05$ as compared to control group. [b]$P<0.05$ as compared to streptozotocin (STZ)-treated diabetic group.

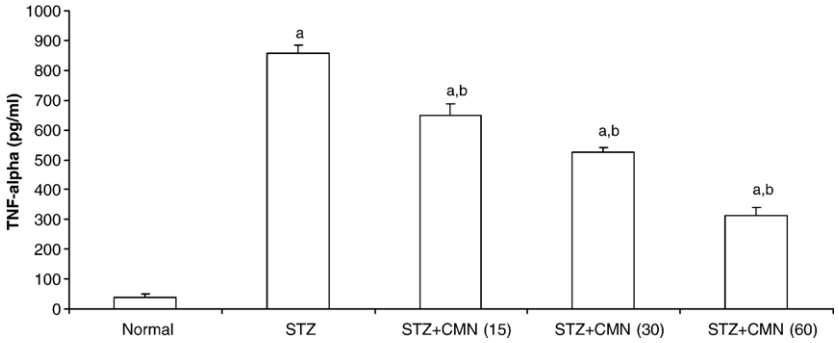

Fig. 3. Effect of curcumin (15, 30 and 60 mg/kg, p.o.) on TNF-α release in control and diabetic mice. CMN (15)=Curcumin, 15 mg/kg, CMN (30)=Curcumin, 30 mg/kg and CMN (60)=Curcumin, 60 mg/kg. Values are expressed as mean±S.E.M. ($n=7$ in each group). [a]$P<0.05$ as compared to control group. [b]$P<0.05$ as compared to streptozotocin (STZ)-treated group.

3.4. Effect of experimental diabetes and curcumin treatment on brain nitrite concentration

A marked increase in brain nitrite levels was observed in diabetic as compared with control mice. Curcumin administration (15, 30 and 60 mg/kg) for 4 weeks starting from 4th week resulted in a significant decrease in the % nitrite levels as compared with diabetic mice ($P<0.05$, Fig. 4).

4. Discussion

In the present study, streptozotocin-injected mice had significantly higher blood glucose levels, decreased body weights and the nociceptive threshold was significantly lower than non-diabetic mice, indicating that diabetic mice exhibit thermal hyperalgesia. This is in line with observation of Ohsawa and Kamei (1999) that streptozotocin-induced mice had thermal allodynia and hyperalgesia tested on exposure of tail to noxious heating. Similar models of mechanical hyperalgesia and formalin-evoked flinching in streptozotocin-induced mice have been demonstrated previously (Ahlgreen and Levine, 1993; Calcutt et al., 1996).

Present studies on tail immersion and hot-plate assays reveal that curcumin, a well-known antioxidant, prevents neuropathic pain in streptozotocin-injected mice. Diabetic neuropathy develops as a result of hyperglycaemia induced local metabolic and microvascular changes. The pathogenesis of diabetic neuropathy is complex and involves multiple pathways. Studies have demonstrated that even with stringent blood glucose control, the prevention of neuropathy is not successful which suggests that there may be a release of early mediators between hyperglycaemia-induced metabolic and enzymatic changes and the nerve damage. Once these mediators are released, it is possible that they modulate neuronal homeostasis independently of the initial metabolic stimulus. These mediators have been proposed to be neuropoietic cytokines such as IL-1, IL-6 and TNF-α (Skundric and Lisak, 2003). Previous studies have shown that chronic hyperglycaemia accelerates the production of endogenous TNF-α in microvascular and neuronal tissues; Thus, TNF-α has been demonstrated as an important mediator of neuropathic pain (Ignatowski et al., 1999).

It has been observed in our study also that curcumin at varying doses reduces the levels of NO and TNF-α, which explains the anti-inflammatory activity of curcumin and gives us an indication that an increased level of NO and cytokine levels are responsible for a decrease in the pain threshold observed in mice. These results correlate with the observation that TNF-α is implicated in the initiation of neuropathic pain

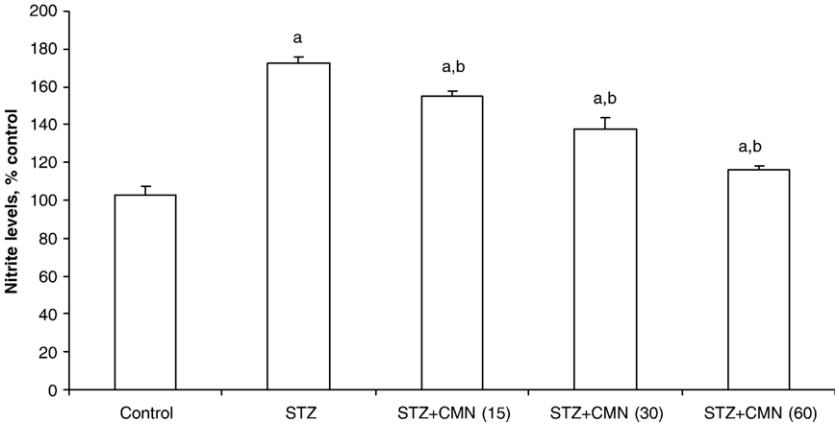

Fig. 4. Effect of curcumin (15, 30 and 60 mg/kg, p.o.) on nitrite levels (% control) in control and diabetic mice. CMN (15)=Curcumin, 15 mg/kg, CMN (30)=Curcumin, 30 mg/kg and CMN (60)=Curcumin, 60 mg/kg. Values are expressed as mean±S.E.M. ($n=7$ in each group). [a]$P<0.05$ as compared to control group. [b]$P<0.05$ as compared to streptozotocin (STZ)-treated group.

which further activates downstream signalling pathway including activation of p38 mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK), and NF-Kb (Masamune et al., 2005). Previous studies also reveal that clinical application of specific agents that suppress production and/or activity of TNF-α and NO may inhibit the development and exacerbation of chronic diabetic complications. Given that only prophylactic treatment with TNF-α inhibitors efficiently reduces hyperalgesia, TNF-α seems to contribute to the initiation of neuropathic pain (George et al., 1999).

Other key mediators of glucose-induced oxidative injury are superoxide and nitric oxide which combine together to form peroxynitrite, which rapidly causes protein nitration or nitrosylation, lipid peroxidation, DNA damage and cell death and has direct toxic effects on the nerve tissue leading to neuropathic pain (Kim et al., 2003). A marked increase in the whole brain nitrite levels was observed in the diabetic animals which indicate nitrosative stress. Curcumin treatment at varying doses attenuated the increased nitrite levels.

Recent studies have shown that curcumin is both a nitric oxide scavenger and an inhibitor of inducible nitric oxide synthase (iNOS) expression, low levels of which correlate with anti-apoptotic function and poor survival which may be regulated by inhibition of NF-κB activation (Nanjii et al., 2003). Curcumin, an inhibitor of NF-κB, ameliorates the surge of pro-inflammatory cytokines during cardiopulmonary bypass and there is a decreased cardiomyocytic apoptosis after global cardiac ischemia/reperfusion injury (Yeh et al., 2005). Curcumin has the ability to inhibit iNOS induction by lipopolysaccharide in the mammary glands and to scavenge NO radicals and reduce TNF-α, which might explain, at least partly, its therapeutic properties in inflammation (Onoda and Inano, 2000). Previous results support the early findings that *N*-acetylcysteine and pentoxifylline, free radical scavengers and inhibitors of tumor necrosis factor-α production, inhibit the development of peripheral neuropathy in streptozotocin-induced diabetic rats (Qiang et al., 1998). Curcumin is also known to reduce the amount of peroxynitrite formed by the reaction between oxygen and nitric oxide, generated from sodium nitroprusside (Sreejayan and Rao, 1997). On a closer scrutiny of the cytokine profile and nitric oxide production by immune cells, it has been shown that there is an initial down-regulation of Th1 cytokine response and NO production treated with curcumin (Bhaumi et al., 2000).

Based on the present preliminary results, we conclude that curcumin is a novel antinociceptive agent and can be used as a therapeutic option in the treatment of neuropathic pain associated with diabetes mellitus. Further studies are warranted to explore the exact mechanism of curcumin's antinociceptive effect.

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