Anti-nociceptive effect of duloxetine in mouse model of diabetic neuropathic pain

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The involvement of adenosinergic pathway in the anti-nociceptive effect of duloxetine, a balanced 5-HT/NE reuptake inhibitor, was evaluated in streptozotocin induced diabetic male albino mice of Laca strain. After four weeks of single injection of streptozotocin (200 mg/kg, ip), mice were tested in the tail immersion and hot-plate assays. Cerebral adenosine levels were measured by high-performance liquid chromatography (HPLC/PDA detector). Diabetic mice exhibited significant hyperalgesia along with increased plasma glucose, decreased body weights and reduced cerebral adenosine levels. Administration of duloxetine (5, 10 and 20 mg/kg, ip) to diabetic mice produced dose-dependent anti-nociceptive effect in both tail-immersion and hot-plate assays. Adenosine levels were also significantly and dose-dependently increased by different doses of duloxetine. The results demonstrated the involvement of adenosinergic pathway in duloxetine mediated anti-hyperalgesia in diabetic neuropathic pain.

Keywords Adenosine, Diabetes, Duloxetine, Neuropathy

Diabetic peripheral neuropathy is the most common complication of long-standing diabetes mellitus which frequently results in clinically significant morbidities e.g. pain, foot ulcers and amputations. Although estimates of diabetic neuropathy vary widely depending on the assessment criteria employed, as many as 50% diabetics have some degree of neuropathic pain. A large number of neuroanatomical, neurophysiologic and neurochemical mechanisms are thought to contribute to the development and maintenance of diabetic neuropathic pain. Hyperglycemia clearly plays a key role in the development and progression of diabetic neuropathy as well as the other microvascular complications of diabetes. Understandably, then, investigations into the molecular and biochemical pathophysiology of diabetic neuropathy have focused on glucose metabolite pathways.

Over the past 25 years, animal experiments and in vitro studies have identified biochemical pathways likely to be important in the development of diabetic complications and have led to possible approaches to treatment. All of these pathways are related to the metabolic and/or redox state of the cell. Pathways which are mainly driven by metabolism are: glucose flux through the polyol pathway; the hexosamine pathway; excess/inappropriate activation of protein kinase C (PKC) isoforms and accumulation of advanced glycation end products. While each pathway may be injurious alone, collectively they cause an imbalance in the mitochondrial redox state of the cell and lead to excessive formation of reactive oxygen species (ROS). Increased oxidative stress within the cell leads to activation of the poly (ADP-ribose) polymerase (PARP) pathway, which regulates the expression of genes involved in promoting inflammatory reactions and neuronal dysfunction.

Although neuronal loss or alteration of neurotransmitters have been reported to be responsible for the changed pain perception, the exact etiologic factors remains unexplored. More recently, Bomholt et al. suggested that antidepressants that variously affect both noradrenaline and serotonin levels have more potent and efficacious antinociceptive effects than selective serotonin reuptake inhibitors (as exemplified by citalopram), against a range of pain-like behaviours in an animal model of neuropathic pain. Other than all other neurotransmitters such as dopamine and serotonin, it is probable that nucleosides and more particularly adenosine can play a role in the pathophysiology of neuropathic pain. Experimental observation
suggested involvement of adenosine in nociceptive effect of amitriptyline in nerve-injury-induced neuropathic pain.\textsuperscript{11}

Duloxetine hydrochloride is a selective 5-HT and NE reuptake inhibitor that is relatively balanced in its affinity for both 5-HT and NE reuptake inhibition\textsuperscript{12} and is the first Food and Drug Administration—approved prescription drug for the management of diabetic neuropathy. In clinical trials, duloxetine has been shown to be safe and effective in the treatment of depression\textsuperscript{13-15} and can significantly reduce painful physical symptoms associated with major depressive disorder.\textsuperscript{16} Based on preclinical\textsuperscript{17} and clinical\textsuperscript{18,19} studies of duloxetine, this compound was tested in an earlier study to explore its effects in humans with diabetic neuropathy.\textsuperscript{18} Jones et al.\textsuperscript{19} reported that duloxetine may be efficacious in the treatment of persistent and/or inflammatory pain states. However, the mechanism of indirect modulation of duloxetine analgesia by other neurotransmitters has not been evaluated in the diabetic neuropathic pain. Therefore, the present study has been designed to explore involvement of adenosinergic pathway, if any, in the protective action of duloxetine in diabetic neuropathic pain in mice.

Materials and Methods

Animals—Male albino mice of Laca strain (22-30 g) bred in Central Animal House facility of Panjab University were used. The animals were housed under standard laboratory conditions, maintained on a 12:12 hr light:dark cycle and had free access to food (Ashirwad Industries, Mohali, India) and water. Animals were acclimatized to laboratory conditions before the tests. All experiments were carried out between 0900 and 1700 hrs. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Panjab University and conducted according to the Indian National Science Academy guidelines for the use and care of animals.

Drugs—Streptozotocin (Sigma; St. Louis, MO, USA) was prepared in citrate buffer (pH 4.4, 0.1 M) and duloxetine (Ranbaxy Research Lab, Gurgaon) was dissolved in saline (0.9%) after triturating with 10% Tween 80. The drugs were administered intraperitoneally in a constant volume of 1 ml/100 g body weight.

Induction and assessment of diabetes—The streptozotocin-induced diabetic mouse model of neuropathic pain was used\textsuperscript{20}. Diabetes was confirmed after 48 hr of streptozotocin injection (200 mg/kg; ip); the blood samples were collected through tail vein and plasma glucose levels were estimated by enzymatic GOD-PAP (glucose oxidase peroxidase) diagnostic kit (Span Diagnostic Chemicals, India.) method. The mice having plasma glucose levels more than 14 mmol/l were selected and used for the present study.\textsuperscript{20}

Treatment schedule—After a basal recording of nociceptive reaction at 4th week of streptozotocin injection, control and diabetic rat were randomly selected and divided in 8 groups of 6-7 animals each. Group 1 was the non-diabetic controls and animals received a single vehicle injection of citrate buffer. Group 2 was the diabetic control. Group 3, 4 and 5 consisted of diabetic animals and received duloxetine 5, 10 and 20 mg/kg respectively. Group 6 was the control animals which received duloxetine (20 mg/kg)

Assessment of thermal hyperalgesia—Tail-immersion (warm water) test: In tail-immersion test, tail of mice was immersed in a warm water bath (52.5°C ± 0.5°C) until tail withdrawal (flicking response) or signs of struggle were observed (cut-off time 12s). Shortening of the tail withdrawal time indicates hyperalgesia and is attributed to central mechanisms.\textsuperscript{21,22}

Hot-plate test: The hyperalgesic response on the hot-plate is considered to result from a combination of central and peripheral mechanisms.\textsuperscript{22} In this test, animals were individually placed on a hot-plate (Eddy's Hot-Plate) with the temperature adjusted to 55°C ±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 sec in order to avoid damage to the paw. In both tail immersion and hot plate assays, nociceptive latency was measured at 15, 30, 60, 120 and 180 min after duloxetine injection and expressed as percent of the maximum possible effect (percent MPE), where percent MPE = (post-drug threshold-pre-drug threshold) × 100 / (cut-off time – pre-drug threshold).

Measurement of adenosine levels in mouse brain by using High Performance Liquid Chromatography (HPLC)—HPLC method using photodiode array (PDA) detector was used to determine the endogenous levels of adenosine in mouse brain, and to investigate any alteration in its level during diabetic neuropathic pain. Waters standard system consisting of a high pressure isocratic pump, a 20 µmol sample injector valve, C18 reverse phase column and PDA detector.
were used. Mobile phase consisted of 0.01 \( M \) sodium dihydrogen phosphate (\( \text{NaH}_2\text{PO}_4 \)) and HPLC grade methanol at a ratio of 85:15; pH of the mobile phase was adjusted to 6.1 with anhydrous sodium carbonate. After behavioral studies animals were sacrificed under deep anaesthesia and brains were isolated and homogenized in 0.1 \( M \) perchloric acid solution and centrifuged at 15000 \( g \) for 15 min. The supernatant was further filtered through 0.25 \( \mu \) filters before injecting into the HPLC injection pump. Samples (20 \( \mu l \)) were injected manually. Data were recorded and analyzed with the help of Empower® software.

Statistical analysis—The nociceptive threshold, i.e. the latency (in seconds) to thermal noxious stimuli was measured and % MPE was calculated. The data are expressed as mean\(\pm\)S.E. The intergroup variation was measured by analysis of variance (ANOVA) followed by Tukey’s test to assess the significance. Statistical significance was considered at \( P<0.05 \). The statistical analysis was done using the Jandel Sigma Stat Statistical Software version 2.0.

Results

Effect of streptozotocin-injection on blood glucose and body weights—After four weeks of streptozotocin injection, diabetic mice exhibited significantly increased plasma glucose levels (22.02\(\pm\)0.36 mmol/l) as compared to non-diabetic mice (6.01\(\pm\)0.85 mmol/l; \( P<0.001 \)). There was a marked decrease in the body weights of STZ-injected mice (22.8\(\pm\)1.46 g) as compared with age matched control mice (26.0\(\pm\)1.56 g, \( P<0.05 \)).

Effect of streptozotocin injection on nociceptive threshold—The nociceptive threshold was significantly lower in diabetic mice as compared with the basal value tested in both the tail immersion and hot-plate assays. Hyperalgesia was evident in the tail-immersion and hot-plate after 1 and 2 weeks respectively, and the maximum decrease in pain threshold was observed at 4 weeks after streptozotocin injection in mice (Table 1).

Effect of duloxetine treatment on nociceptive threshold in control and streptozotocin-induced diabetic mice—Systemic administration of duloxetine (5, 10 and 20 mg/kg) produced a significant and dose-dependent increase in % MPE in both the tail immersion and hot-plate assays as compared to untreated diabetic mice (Fig. 1). The percent MPE produced by duloxetine (20 mg/kg) was significantly lower in diabetic mice than in the control mice. The maximum percent MPE was observed at 60 min after administration of duloxetine.

Effect of duloxetine treatment on brain adenosine levels in control and streptozotocin-induced diabetic mice—Diabetic mice showed significant decrease (80\(\pm\)0.79 ng mg\(^{-1}\) brain tissue) in brain adenosine levels as compared to control mice (108\(\pm\)1.5 ng mg\(^{-1}\)).

Table 1—General parameters in streptozotocin-induced diabetic mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Basal</th>
<th>1 week</th>
<th>2 week</th>
<th>3 week</th>
<th>4 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td>26 (\pm) 24.1 (\pm) 23.8 (\pm) 23.0 (\pm) 22.8 (\pm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail-Fick (s)</td>
<td>1.56</td>
<td>1.39</td>
<td>1.73(^a)</td>
<td>1.25(^a)</td>
<td>1.46(^a)</td>
</tr>
<tr>
<td>Hot-Plate (s)</td>
<td>4.27</td>
<td>2.57(^b)</td>
<td>1.83(^b)</td>
<td>1.46(^b)</td>
<td>1.34(^b)</td>
</tr>
</tbody>
</table>

\(^a\)\( P<0.001 \) as compared with corresponding basal values
Adenosine is an endogenous vasodilator, which is functionally implicated in neuronal disorders and is proposed to be an endogenous neuroprotective agent. Adenosine and adenosine receptor agonists have antinociceptive effect in animal model of acute inflammation and nerve injury-induced neuropathic pain. Systemic administration of adenosine and adenosine receptor agonists was reported to produce analgesic effects in diabetic patients with neuropathic pain. Furthermore, a significant reduction in levels of adenosine in cerebrospinal fluid of patients with neuropathic pain or hyperglycemia has been reported. Adenosine kinase inhibitors by enhancing adenosine levels, show neuroprotective effects in animal models of neuropathic pain and theophylline pretreatment enhance neuronal death under hyperglycemic situation resulting in greater ischemic injury. Acute hyperglycemia was reported to decrease the levels of adenosine and this could be responsible for its ischemic effects, hence, the beneficial effects of exogenous adenosine administration. Saini et al. reported that adenosine significantly attenuated the effects of acute hyperglycemia on nerve condition velocity and nerve blood flow, and suggested that adenosine analogues could have beneficial effects in prevention of diabetic neuropathy. Therefore, interaction with the endogenous adenosinergic system appears to be the most important for the antinociceptive action of duloxetine. In the present study, there was significant decrease in the cerebral adenosine levels of the diabetic mice as compared to control mice. Since, adenosine can cause inhibition of neuronal excitability; it is probable that a deficit in this nucleoside can result into spontaneous occurrence of pain messages. This can easily be explained by the behavioral studies, as shown by the decrease in pain threshold in both tail immersion and hot plate test. Low adenosine levels are associated with induction of spontaneous neuropathic pain. Administrations of duloxetine resulted in significantly and dose-dependently increase in cerebral adenosine levels, which can also be easily correlated to its analgesic action. Even the minimal increase in adenosine levels, would be sufficient to afford considerable pain relief, explained by alleviation of neuropathic pain at low dose of adenosine. Earlier, Ulugol et al. reported anti-allodynic effect of both peripheral and systemic amitriptyline, and suggested the involvement of endogenous adenosine in the action of amitriptyline in the rat model of painful diabetic neuropathy. Thus, the findings of this study further strengthen the view of interaction between adenosine and duloxetine in attenuation of diabetic neuropathic pain.

Table 2—Effect of duloxetine on whole brain adenosine levels in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adenosine levels (ng mg⁻¹ brain tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>108 ± 1.5</td>
</tr>
<tr>
<td>Diabetic mice</td>
<td>80 ± 0.79a</td>
</tr>
<tr>
<td>Diabetic + duloxetine (5 mg/kg)</td>
<td>130 ± 2.4b,c</td>
</tr>
<tr>
<td>Diabetic + duloxetine (10 mg/kg)</td>
<td>142 ± 3.1b,c</td>
</tr>
<tr>
<td>Diabetic + duloxetine (20 mg/kg)</td>
<td>149 ± 4.0b,c</td>
</tr>
<tr>
<td>Control + duloxetine (20 mg/kg)</td>
<td>168 ± 2.5b,c</td>
</tr>
</tbody>
</table>

P values: <0.05; as compared to a control group, b diabetic group; c one another (ANOVA followed by Tukey’s test)
Acknowledgement

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References