Resveratrol, a polyphenolic phytoalexin attenuates thermal hyperalgesia and cold allodynia in STZ-induced diabetic rats

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The effects of resveratrol, a polyphenolic phytoalexin present in red wine have been investigated on hyperalgesia and cold allodynia in streptozotocin (STZ) induced diabetic rats. Diabetes was induced by a single intraperitoneal injection of streptozotocin (65mg/kg). After 4-weeks of STZ injection, diabetic rats exhibited a significant thermal hyperalgesia and cold allodynia along with increased plasma glucose and decreased body weights as compared with controls rats. Chronic treatment with resveratrol (10mg/kg orally) from week 4 to week 6 significantly attenuated the cold allodynia and thermal hyperalgesia. The results emphasize the role of oxidative stress in development of hyperalgesia and cold allodynia in diabetic animals and point towards the potential of resveratrol as an adjuvant therapy for the prevention and treatment of diabetic neuropathy.

Keywords: Diabetic neuropathy, Hyperalgesia, Oxidative stress, Resveratrol.

Diabetic neuropathy is one of the most frequent peripheral neuropathies associated with hyperalgesia, hyperesthesia and cold allodynia. Enhanced oxidative stress plays a significant role in the etiology of neuropathic pain. Resveratrol (3,4',5-trihydroxystilbene), a naturally occurring phytoalexin is present in dietary sources including red wine and is produced in response to injury or fungal infection1,2. Phytoalexins are chemical substances produced by plants as a defence against infection by pathogenic microorganisms such as fungi. The anti-inflammatory effects of resveratrol have been well documented but the mechanism is poorly characterized. A high concentration of NO is produced by inducible NO synthase (iNOS) in inflammation and the prevention of its expression may be an important anti-inflammatory mechanism3. Resveratrol is known to prevent atherosclerosis4, inhibit the proliferation of a number of cell lines and act as a chemo preventive agent also5,6. It reduces the synthesis of lipids in rat liver and inhibits the production of proatherogenic eicosanoids by human platelets and neutrophils7. Resveratrol is known to exert a strong inhibitory effect on superoxide anion and hydrogen peroxide production by macrophages stimulated by lipopolysaccharide or phorbol esters. It has hydroxy-radical scavenging activity and has recently been found to possess glutathione-sparing activity also8. Based on the anti-inflammatory and antioxidant properties of resveratrol, the present study has been designed to evaluate the effect of resveratrol on thermal hyperalgesia and cold allodynia, the indices of diabetic neuropathic pain in rats.

Materials and Methods

Animals—Male Sprague-Dawley rats (200-250 g) bred in Central Animal House facilities of Panjab University were housed under optimal laboratory conditions. Animals were acclimatized to laboratory conditions before the experiments that were carried out between 0900 and 1700 hrs. Approval of the Institutional Animal Ethics Committee was obtained.

Treatment schedule—After a basal reading at 4th week of streptozotocin, (STZ) injection, control and diabetic rats were randomly selected and divided in three groups of 6-7 animals each i.e. control, diabetic control and diabetic group treated with resveratrol. Starting from 4th week till 6th week, the control and diabetic control groups received vehicle of resveratrol and another diabetic treated group received suspension of resveratrol (10mg/kg/day) orally. Resveratrol (Sigma, St.Louis, Mo, USA) suspension was prepared in 0.5% carboxy methyl cellulose solution. All these drugs were administered in a constant volume of 0.5ml/100g body weight of rat.
Induction and assessment of diabetes—A single dose of 65mg/kg body weight STZ prepared in citrate buffer (pH 4.4, 0.1M) was injected intraperitoneally⁹. The age matched control rats received citrate buffer and used along with diabetic animals. Diabetes was confirmed after 48 hr of STZ 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 (Span Diagnostic Chemicals, India)¹⁰. The rats having plasma glucose levels more than 250mg/dl were selected and used for the present study¹¹. Body weight and plasma glucose level were also measured before and at the end of the experiment to see the effect of resveratrol on these parameters.

Assessment of thermal hyperalgesia and cold allodynia—Tail-immersion (warm water) test: The rat tails were immersed in a warm water bath (47°C ± 1°C) until tail withdrawal (flicking response) or signs of struggle were observed (cut-off time 15 sec). Shortening of the tail withdrawal time indicates hyperalgesia.

Paw withdrawal test for rats—In this test, rat paw was immersed in warm water bath (47°C ± 1°C) until paw withdrawal sign was observed (cut-off time 10 sec). The shortening of the paw withdrawal time indicates hyperalgesia¹².

Tail immersion (cold water) test—The temperature of the water was set at 10°C ± 0.5°C, a temperature that is normally innocuous¹³. The shortened duration of tail immersion indicates allodynia. The cut-off time was 15 sec.

Statistical analysis—Results are expressed as mean ± SE. Increase in tail and paw withdrawal latency (in seconds) in hyperalgesic and cold allodynia responses respectively were subjected to analysis of variance (ANOVA) followed by Dunnett’s t-test to assess the significance. P<0.05 was considered statistically significant. Unpaired Student’s t-test was used to compare the differences between two groups.

Results

Blood glucose and body weights—Four weeks after STZ injection, diabetic animals exhibited significantly increased blood glucose levels (394.5 ± 12.23 mg/dl) than control rats (125.30 ± 11.24 mg/dl, P<0.05). There was a marked decrease in the body weight in the STZ-injected rats (162 ± 2.23g) compared with control rats (231.25 ± 8.94g, P<0.05).

Effect of chronic resveratrol treatment on diabetic pain threshold in tail-immersion and paw withdrawal (warm water) tests—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). Resveratrol administration for 2 weeks starting from 4th week significantly increased the tail withdrawal and paw withdrawal latencies from 4th to 6th week compared to control diabetic rats (P<0.05, Fig.1A and C).

Effect of chronic resveratrol treatment on diabetic pain threshold in tail immersion (cold water) test—At the end of the 4th week, diabetic animals exhibited decrease in pain threshold from non-noxious stimuli as compared to control rats (P<0.05). Resveratrol administration for 2 weeks starting from 4th week significantly increased the tail withdrawal latencies up to 6th week compared to control diabetic rats (P<0.05, Fig.1B).

Fig. 1—Effect of chronic treatment of resveratrol on pain threshold values in STZ-injected diabetic rats subjected to the tail immersion test in (A) warm water (47°C±1°C), (B) cold water (10°C±0.5°C) and (C) paw withdrawal test in warm water (47°C±1°C). Values are expressed in mean ± SE. *P< 0.05; compared to “control; #diabetic control at respective weeks.
Discussion

A marked decrease in nociceptive threshold in STZ-injected diabetic rats as compared with control rats indicates development of significant hyperalgesia and cold allodynia in diabetes. Similar models of mechanical hyperalgesia, thermal allodynia and formalin-evoked flinching in STZ-rats have been previously demonstrated\textsuperscript{14,15}. Diabetic neuropathy is one of the most frequent peripheral neuropathies associated with hyperalgesia and hyperaesthesia. Strong evidence implicates oxidative stress as a mediator of diabetes-induced microvascular complications, including distal symmetric polyneuropathy\textsuperscript{16}. Key mediators of glucose-induced oxidative injury are superoxide anions and nitric oxide (NO)\textsuperscript{17}. Besides alteration of the neuronal nitric oxide synthase (nNOS), alteration in the levels of neurotransmitters, is a key factor in the pathogenesis of diabetic neuropathy\textsuperscript{16}. Superoxide ions are believed to underlie many of the oxidative changes in hyperglycaemic conditions, including increase in aldose reductase and protein kinase C activity. Superoxides can also react with NO, forming peroxynitrite (ONOO\textsuperscript{-}), which rapidly causes protein nitration or nitrosylation, lipid peroxidation, deoxyribonucleic acid (DNA) damage, and cell death. Peroxynitrite formation is dependent on both superoxide and NO concentrations, therefore, cells that constitutively express NO synthase, such as endothelial cells and neurons, may be more vulnerable to peroxynitrite induced cell death in conditions favouring the production of superoxides\textsuperscript{19}. The dorsal root ganglia neurons are also susceptible to glucose-mediated oxidative stress and die by apoptotic mechanisms in animal and cell culture models of diabetes\textsuperscript{20}.

Present findings on tail immersion (noxious and non-noxious) and paw withdrawal methods indicate that resveratrol at least prevents spinal neuropathy and hyperalgesia in diabetic rats. This is in agreement with the reports where antioxidants like glutathione (GSH) and alpha-lipoic acid, significantly prevented thermal and mechanical hyperalgesia. Resveratrol strongly inhibits NO generation in activated macrophages, as measured by the amount of nitrite released into the culture medium and resveratrol markedly reduced the amount of cytosolic iNOS protein and steady state mRNA levels\textsuperscript{21}. It was also reported that the Ca\textsuperscript{2+}-dependent activities of membrane-associated PKC alpha induced by either phorbol ester or diacylglycerol were potently inhibited by resveratrol\textsuperscript{22,23}. Impaired blood flow also seems to contribute to noxious stimulus hypersensitivity and vasodilator treatment has been demonstrated to reduce allodynia in diabetic rats. There is some evidence that resveratrol interacts with the vascular NO system. Resveratrol caused relaxation of the phenylephrine-precontracted rat aorta, an effect that was endothelium dependent and mediated by NO\textsuperscript{24}. Thus, resveratrol may have improved neuronal blood flow through a scavenging activity on reactive oxygen species or by its direct vasorelaxant properties.

In conclusion, the use of polyphenols (antioxidants) such as resveratrol may prove as a potential candidate for treating diabetic neuropathic pain and other diabetic complications.

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References


