

Involvement of p38 MAPkinase in attenuation of antinociceptive effect of morphine in diabetic mice

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Experimental diabetes induced by streptozotocin (200 mg/kg, ip) markedly decreased the antinociceptive effect of morphine and significantly increased the urinary nitrite concentration. Administration of FR-167653 (a selective p38MAPKinase inhibitor) in a dose of 4 mg/kg improved the antinociceptive effect of morphine and attenuated the increase in urinary nitrite concentration in diabetic mice. It may be concluded that diabetes-induced decrease in antinociceptive effect of morphine may be due to induction of p38 MAPKinase activity.

Keywords: Antinociception, Diabetes, FR-167653, Morphine, p38MAPKinase

Experimental diabetes has been reported to decrease antinociceptive effect of morphine^{1,2}. Expression of immunoreactive cytokines in diabetic nerves has been implicated in the pathogenesis of diabetic neuropathy^{3,4}. Cytokines like TNF- α , IL-1 and IFN- γ significantly increase p38 mitogen-activated protein kinase (MAPKinase) activity^{5,7}. FR-167653 selectively inhibits the activity of p38 mitogen-activated protein kinase (MAPKinase) without influencing the activity of other kinases^{8,9}. Therefore, in the present study, FR-167653 has been used to explore the involvement of p38MAPKinase in decreased antinociceptive effect of morphine in diabetic mice.

Materials and Methods

Animals—Swiss albino mice (20-30g) of either sex were employed in the present study. Animals were housed in institutional animal house under standard conditions, with 12 hr light / dark cycle and they had free access to food (Kisan Feeds Ltd., New Delhi, India) and tap water. Experimental protocol was approved by Institutional Animals Ethics Committee (IAEC).

Estimation of nociceptive threshold—The nociceptive threshold in mice was determined using withdrawal latency in TailFlick test¹⁰ using Tail-Flick analgesiometer (INCO Pvt. Ltd., Ambala, India). The tail of each mouse was exposed to radiant heat given by a nichrome wire. The intensity of the radiant heat was adjusted to obtain a basal or pre-treatment latency of 2-4 seconds in both diabetic and non-diabetic animals. Tail-Flick latency is the time interval taken by mouse to flick its tail after exposure to a source of radiant heat. Cut off latency was fixed at 10 seconds. Tail - Flick latency was expressed as percentage of the maximum possible effect (% MPE):

$$\text{MPW (\%)} = \frac{\text{Post - treatment - Pre - treatment latency}}{\text{Cut off time - Pre - treatment latency}} \times 100$$

Pre-treatment latency refers to the control latency before drug administration, while post-treatment latency refers to the latency after drug administration.

Estimation of plasma glucose levels—Blood was withdrawn from tail vein of mice and plasma was extracted using cooling centrifuge at 2500 r.p.m. for 10 min. Plasma glucose levels was estimated colorimetrically at 500 nm by Glucose-oxidase method¹¹ using a commercially available enzymatic kit (CDR Medical Industries Ltd., Hyderabad, India). Mice with fasting plasma glucose levels of more than 250 g/dl were considered to be diabetic and were included in the study.

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Estimation of urinary nitrite—Each mouse was paced individually in a metabolic cage and its urine was collected for 24 hr. The animals were allowed to drink water *ad libitum* before the study but were denied any water during the 24 hr study period. Nitric oxide (NO) is largely converted to nitrite in the presence of oxygen, water and haemoglobin¹². Urinary nitrite was estimated using Greiss reagent¹³, which served as an indicator of NO production. Greiss reagent (2.0 ml; 1% sulphanilamide and 0.1% naphthylethylene diamine in 5% phosphoric acid) was added to 2.0 ml of suitably diluted urine and optical density was measured at 550 nm (Spectrophotometer, Beckman DU 640-B, Nyon, Switzerland). Nitrite concentration was calculated using a standard curve for sodium nitrite. Nitrite levels were expressed as the absolute amount excreted in 24 hr.

Drugs—Streptozotocin (Pharmacia and Upjohn, Kalamazoo, USA) was dissolved in 0.1 N citrate buffer. Morphine (Jackson Labs., Amritsar, India) was dissolved in distilled water. FR-167653 (Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) was dissolved in saline.

Drug protocol—Mice were divided into 6 groups of 5 each.

Vehicle treated groups (Groups I - II)—Non-diabetic and diabetic mice were injected with vehicle (normal saline) for 7 days.

FR-167653 treated groups (Groups III - VI)—Age matched non-diabetic and diabetic mice were administered FR-167653 (2 mg/kg, ip or 4 mg/kg, ip) four times daily for 7 days.

At the end of four days and seven days treatment, mice in all the groups were injected with morphine (10 µg dissolved in 5 µl distilled water) by intracerebroventricular injection under light ether anaesthesia¹⁴. Mice were subjected to Tail - Flick Test immediately, 5, 15, 30, 45, 60, 90 and 120 min after morphine administration.

Statistical analysis—All the results are expressed as mean±SE. Data were analyzed by One-way analysis of variance (ANOVA) followed by the Studentized Range Procedure for 5% allowance. $P < 0.05$ was considered as significant.

Results

Effect of experimental diabetes on antinociceptive effect of morphine—Streptozotocin-induced diabetes significantly attenuated the morphine-induced increase in % MPE as compared to that of non-diabetic mice.

Effect of FR-167653 treatment on antinociceptive effect of morphine—Administration of FR-167653 (2 mg/kg, ip, q.i.d. or 4 mg/kg, ip, q.i.d.) for 4 or 7 days did not produce any effect per se on antinociceptive effect of morphine in non-diabetic mice (Fig. 1). Administration of FR-167653 in low dose (2 mg/kg, ip, q.i.d.) for four days produced no change in the antinociceptive effect of morphine in diabetic mice (Fig. 1). However, FR-167653 treatment in low dose (2 mg/kg) for 7 days attenuated the decreased antinociceptive effect of morphine in diabetic mice. On the other hand, high dose (4 mg/kg, ip, q.i.d.) FR-167653 treatment for 4 or 7 days significantly attenuated diabetes-induced decrease in antinociceptive effect of morphine (Fig. 1).

Effect of experimental diabetes on urinary nitrite—Experimental diabetes increased the urinary nitrite concentration.

Effect of FR-167653 treatment on urinary nitrite—Administration of FR-167653 in low as well as high doses produced no change in urinary nitrite concentration in control or non-diabetic mice. On the other hand, low dose (2 mg/kg) treatment of FR-167653 for 7 days markedly reduced the diabetes-induced increase in urinary nitrite concentration. High dose (4 mg/kg) treatment of FR-167653 for 4 or 7 days significantly reduced the diabetes-induced increase in urinary nitrite concentration (Fig. 2).

Discussion

The results of the present study confirm the earlier observation that diabetes attenuates the antinociceptive effect of morphine^{1,2}. However, the mechanism of this decreased antinociceptive effect of

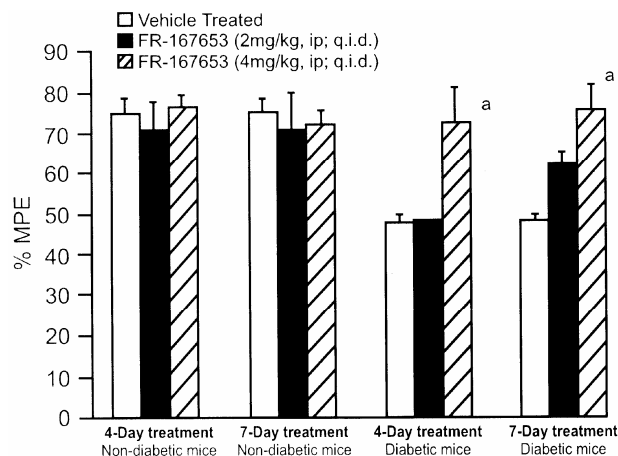


Fig. 1—Effect of FR-167653 treatment on antinociceptive effect of morphine in non-diabetic and diabetic mice (values are mean ± SE for 5 animals in each group. ^a $P < 0.05$ vs non-diabetic mice)

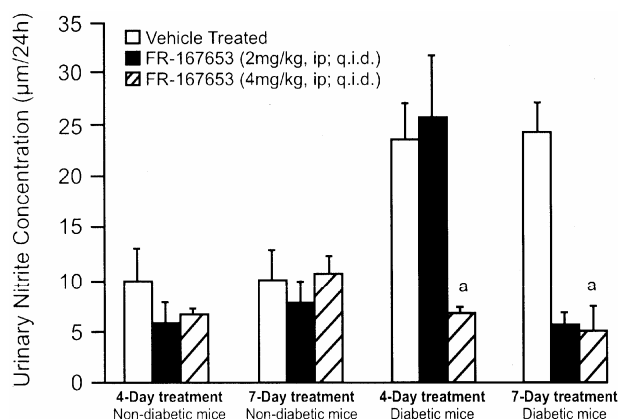


Fig. 2—Effect of FR-167653 treatment on urinary nitrite concentration in non-diabetic and diabetic mice (values are mean \pm SE for 5 animals in each group. ^a $P < 0.05$ vs non-diabetic mice)

morphine as a consequence to diabetes is not known. Increased nitric oxide formation may be responsible for the noted attenuation of antinociceptive effect of morphine in diabetic mice¹⁵. Cytokines are reported to induce nitric oxide synthase expression through the activation of p38 MAPKinase^{6,7,16}. In the present study, FR-167653, a p38 MAPKinase inhibitor restored the antinociceptive effect of morphine and simultaneously reduced diabetes-induced increase in urinary nitrite concentration. Therefore, it may be suggested that diabetes-induced attenuation of antinociceptive effect of morphine may be due to activation of p38 MAPKinase and a consequent increase in nitric oxide formation.

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