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Original Article

ATTENUATION OF ANTINOCICEPTIVE EFFECT OF MORPHINE IN DIABETIC MICE: NITRIC OXIDE OR INTERLEUKIN-2

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ABSTRACT

Objective: The present study was designed to explore the mechanistic role of interleukin-2 in diabetes-induced decrease in the antinociceptive effect of morphine in mice. Role of interleukin-2 was investigated by employing cyclosporin, a interleukin-2 synthesis inhibitor.

Methods: Diabetes was induced in mice by single intra peritoneal injection of Streptozotocin (200 mg/kg, i. p.). Nociceptive threshold in diabetic mice was measured by Rodent tail-flick test. Nitrite levels in the urine of mice were estimated by employing Greiss reagent.

Results: A significant decrease in antinociceptive effect of morphine was observed in mice. Administration of cyclosporin (20 mg/kg, s. c., b. d.) in diabetic mice significantly increased antinociceptive effect of morphine in diabetic mice. However, administration of cyclosporin (20 mg/kg, s. c., b. d.) failed to significantly change the increased nitrite levels in diabetic mice.

Conclusion: The present study indicates that interleukin-2 may be responsible for decrease in antinociceptive effect of cyclosporine. The study also indicates that the increase in levels of interleukin-2 is independent of an increase in nitrite levels. It may, therefore, be concluded that nitric oxide has no role in nociceptive changes made by interekin-2 in diabetic mice.

Keywords: Analgesic, Cyclosporin, Diabetes, Hyperalgesia.

INTRODUCTION

Diabetic neuropathy develops as a result of prolonged hyperglycemia-induced local metabolic and micro vascular changes and affects peripheral, central, and visceral sensorimotor and motor nerves [1]. Diabetes-induced neuropathic pain is less sensitive to traditional analgesics, including morphine [2, 3]. Antinociceptive effect of morphine is reported to be significantly reduced in diabetic rodents [4, 5]. Metabolic changes induced by hyperglycemia lead to dys regulation of cytokine control and their enhanced expression in diabetic nerves leading to diabetic neuropathy [6]. Diabetic nerves show increased expression of immunoreactive cytokines during the course of diabetic neuropathy, a long term diabetic complication [7]. A significant association between type 1 diabetes and a cytokine; interleukin-2 (IL-2) has been documented in mice [8, 9]. Rapamycin, a blocker of IL-2 receptor signaling resulted in beneficial effects in long term type-1 diabetes [9, 10]. Cyclosporin (20 mg/kg), IL-2 synthesis inhibitor, enhanced antinociceptive effect of morphine in non-diabetic as well as diabetic mice [11, 12].

Nitric oxide (NO), derived from immune reactive cytokines like TNF-α and IL-1 has been implicated to underlie the noted attenuation of antinociceptive effect of morphine [4, 12]. Inducible nitric oxide synthase (iNOS) is transcriptionally regulated by cytokines and produces large amounts of NO for prolonged periods of time. Once expressed, no regulatory mechanism is known for the activity of iNOS [13]. FR-167653, inhibitor of TNF-alpha and IL-1 has been observed to increase antinociceptive effect of morphine in diabetic mice [14]. Amino guanidine (iNOS inhibitor) improved diabetes-induced attenuation of antinociceptive effect of morphine and significantly prolonged the tail-flick latency of mice, in heat radiation tail-flick assay [15, 5].

Therefore, author found it interesting to explore the possible relationship between IL-2 and nitric oxide and hypothesized a possible relation between IL-2 and NO, their observed involvement in altered antinociceptive effect of morphine in diabetic mice.

MATERIALS AND METHODS

Animals

Swiss albino mice (20-30 g) of either sex were employed in the present study. Animals were housed in institutional animal house under standard conditions, with 12 h light / dark cycle and had free access to food and tap water. Experimental protocol was approved by an Institutional Animals Ethics Committee (IAEC). All experiments were conducted in accordance with the CPCSEA guidelines directed by Ministry of Environment and Forests, Government of India.

Experimental protocol

Mice were divided into 6 groups comprising 6 animals each (n = 6). Diabetes was induced in mice by a single intraperitoneal injection of the high dose (200 mg/kg) streptozotocin. The dose of STZ employed in the present study has been reported to be suitable for Swiss albino mice to induce neuropathic symptoms to study thermal hyperalgesia as investigated in the present study. Further, SHD-STZ (single high dose streptozotocin) mouse model has been reported to exhibit robust and reliable neuropathic changes [16-18]. Mice with fasting plasma glucose levels of more than 250 mg/dl were considered to be diabetic and were included in the study. Group I and II consisted of non-diabetic and diabetic mice, which were injected with vehicle for seven days respectively. Group III and IV consisted of non-diabetic and diabetic mice, which were treated with cyclosporin (10 mg/kg, s. c., b. d.) for seven days. Group V and VI consisted of non-diabetic and diabetic mice, which were treated with cyclosporin (20 mg/kg, s. c., b. d.) for seven days. In each cyclosporin treated group, morphine (10 µg/5 µl distilled water, i. c. v.) was administered on fourth day and on seventh day. After each morphine administration on fourth and seventh day, mice were subjected to tail-flick test at 0, 5, 15, 30, 45, 60, 90 and 120 min after morphine administration [21]. For estimation of urinary nitrite levels, urine samples of mice were collected at the end of the fourth and seventh day of cyclosporin (10 or 20 mg/kg, s. c., b. d.) treatments. Each mouse was placed individually in a metabolic cage for 24 h and its urine was collected immediately on completion of 24 h. Urine samples, thus collected, then were analyzed for quantification of nitrite levels by using Greiss reagent.

Drugs and treatments

Streptozotocin (Pharmacia and Upjohn, Kalamazoo, USA), morphine (Jackson Laboratories, Amritsar, India) and cyclosporin (Cipla Ltd., India) were employed in the present study. All other chemicals and reagents employed were of analytical grade.

Estimation of plasma glucose levels

Blood was withdrawn from tail vein of mice and plasma was extracted using cooling centrifuge at 2500 rpm for 10 min. Plasma glucose levels were estimated by Glucose oxidase method [19].

Estimation of nociceptive threshold

The nociceptive threshold in mice was determined by measuring tail flick latency in tail-flick test [20]. Tail flick latency i. e. time taken by mouse to withdraw its tail after exposure to radiant heat, was measured. Cut off latency were fixed at 10 s. Effect of drug treatments on tail flick latency was expressed as percentage of the maximum possible effect (% MPE):

MPE (%) = post-treatment latency–pre treatment latency X 100

Cut off time–pre treatment latency

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

Estimation of urinary nitrite

Urinary nitrite levels were estimated using Greiss reaction, which served as an indicator of NO production.

Statistical analysis

The Statistical analysis of data was performed by one-way ANOVA followed by Tukey's Post hoc test, by employing Graph pad In stat; version 3.05 (Graph Pad Software, San Diego, CA, USA).

RESULTS

Effect of cyclosporin treatment on antinociceptive effect on morphine

Administration of cyclosporin (10 or 20 mg/kg, s. c., b. d.) for four or seven days did not alter the antinociceptive effect of morphine in non-diabetic mice as compared to vehicle treated control mice F (5, 30) = 7.14; *P* = 0.0002 [fig. 1: four day treatment]; F (5, 30) = 3.11; *P* = 0.022 [fig. 2: seven day treatment]. Similarly, in diabetic mice, administration of cyclosporin (10 mg/kg, s. c., b. d.) for four and seven days did not significantly alter the antinociceptive effect of morphine in diabetic mice as compared to vehicle treated diabetic mice F (5, 30) = 7.14; *P* = 0.0002 [fig. 1: four day treatment]; F (5, 30) = 3.11; *P* = 0.022 [fig. 2: seven day treatment].

On the other hand, administration of cyclosporin (20 mg/kg, s. c., b. d.) for four as well as seven days significantly increased the antinociceptive effect of morphine in diabetic mice as compared to vehicle-treated diabetic mice F (5, 30) = 7.14; *P* = 0.0002 [fig. 1: four day treatment]; F (5, 30) = 3.11; *P* = 0.022 [fig. 2: seven day treatment].

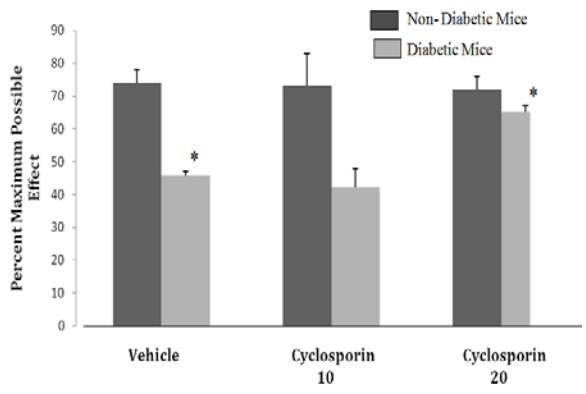

Fig. 1: Effect of four day Cyclosporin treatment on percent Maximum Possible Effect (% MPE) of morphine in non-diabetic and diabetic mice. Data are expressed as mean±S.E.M. n = 6. Data was analyzed by One way ANOVA followed by Tukey's Post hoc Test, F (5, 30) = 7.14; *P* = 0.0002; Values mentioned are in mg/kg, s. c., b. d. *P<0.05 significantly different as compared to non-diabetic mice

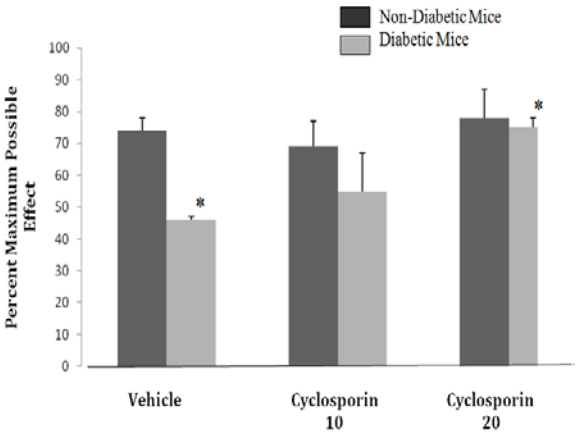

Fig. 2: Effect of seven day Cyclosporin treatment on percent Maximum Possible Effect (% MPE) of morphine in non-diabetic and diabetic mice. Data are expressed as mean±S.E.M. n = 6. Data was analyzed by One way ANOVA followed by Tukey's Post hoc Test, *P* = 0.022; F (5, 30) = 3.11; Values mentioned are in mg/kg, s.c., b.d. *P<0.05 significantly different as compared to non-diabetic mice

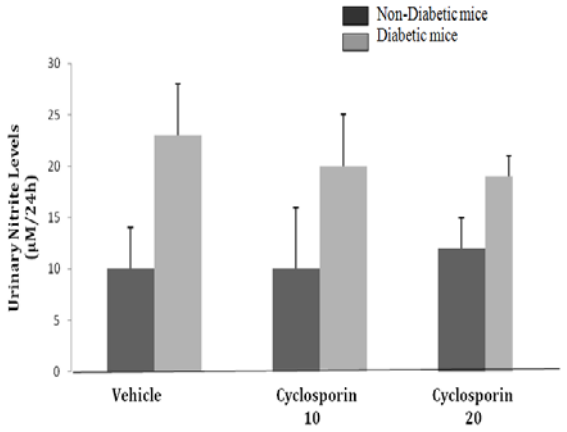

Fig. 3: Effect of four day Cyclosporin treatment on urinary nitrite levels (µM/24h) in non-diabetic and diabetic mice. Data are expressed as mean±S.E.M. n = 6. Data was analyzed by One way ANOVA followed by Tukey's Post hoc Test, *P* = 0.16; F (5, 30) = 1.68. Values mentioned are in mg/kg, s.c., b.d.

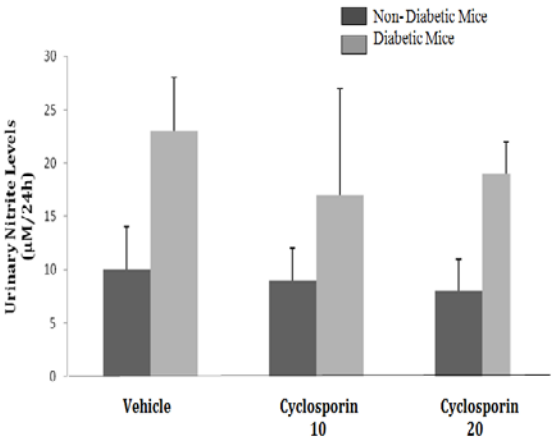

Fig. 4: Effect of seven day Cyclosporin treatment on urinary nitrite levels (µM/24h) in non-diabetic and diabetic mice. Data are expressed as mean±S.E.M. n = 6. Data was analyzed by One way ANOVA followed by Tukey's Post hoc Test, *P* = 0.26; F (5, 30) = 1.36; Values mentioned are in mg/kg, s.c., b.d.

Effect of cyclosporin treatment on urinary nitrite levels in mice

Administration of cyclosporin (10 or 20 mg/kg, s.c., b.d.) for four or seven days did not produce any effect on urinary nitrite levels in non-diabetic mice as well as diabetic mice F (5, 30) = 1.68; *P* = 0.16 [fig. 3: four day treatment]; F (5, 30) = 1.36; *P* = 0.26 [fig. 4: seven day treatment].

DISCUSSION

The results of the present investigation indicated and strengthened the earlier observations that cyclosporin, via inhibition of IL-2, can enhance the antinociceptive effect of morphine in diabetic mice [4, 11]. Results from clinical trials have indicated that interleukin-2 administration is associated with marked toxicity, which limits the quantity of this cytokine that can be administered [22, 23]. Cyclosporin, in a same dose as employed in the present study (20 mg/kg), showed a similar increase in the nociception threshold in the expression phase of morphine tolerance [12]. However, from a mechanistic standpoint, low dose IL-2 treatment has been shown to offer a long term protection from diabetes [24]. Conversely, high doses of IL-2 enhanced immune responses and exacerbated autoimmunity in the NOD mouse, therefore, therapeutic efficacy of IL-2 can vary dramatically depending upon the dose [24]. The observed attenuation of antinociceptive effect of morphine in the present study suggests that severity of diabetes induced in the present study (28 days of streptozotocin injection), may provide a sufficient enough to induce hyperalgesia. Further, observed efficacy of cyclosporin to enhance the antinociceptive effect of morphine indicates induction of large amounts of IL-2 during long exposure (28 days) to streptozotocin, as utilized in the present study. However, quite interestingly, in the present study, cyclosporin did not produce any significant change in diabetes-induced increase in urinary nitrite levels. This observation indicates that unlike other cytokines (TNF-alpha, interleukin-1 or interferon γ); involvement of IL-2 in diabetes-induced attenuation of antinociceptive effect of morphine may be independent of induction of nitric oxide. The observations that involvement of IL-2 in diabetes-induced attenuation of antinociceptive effect of morphine may be independent of nitric oxide levels, unlike other reported cytokines. Further, in a suggesting study, IL-2 has been observed not to increase production of NO [25]. Moreover, NO has been reported to stimulate the synthesis of IL-2 [26].

CONCLUSION

Observations made in the present study decline any possible induction of nitric oxide by interleukin-2 in diabetic mice and indicate a nitric oxide-independent action of IL-2 in attenuation of antinociceptive effect of morphine in diabetic mice.

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ABBREVIATION

IL-2: Interleukin-2, MPE: Maximum Possible Effect, NOS: Nitric Oxide Synthase, NO: Nitric Oxide.

CONFLICT OF INTERESTS

The authors have none conflict of interest

REFERENCES

1. Skundric DS, Lisak RP. Role of neuropoietic cytokines in development and progression of diabetic polyneuropathy: from glucose metabolism to neurodegeneration. Exp Diab Res 2003;4:303–12.
2. Raz I, Hasdi D, Seltzer Z, Melmed RN. Effect of hyperglycemia on pain perception and on efficacy of morphine analgesia in rats. Diabetes 1988;37:1253.
3. Williams J, Haller VL, Stevens DL, Welch SP. Decreased basal endogenous opioid levels in diabetic rodents: effects on morphine and delta-9-tetrahydrocannabinoid-induced antinociception. Eur J Pharmacol 2008;584:78-86.
4. Grover VS, Sharma A, Singh M. Role of nitric oxide in diabetes-induced attenuation of antinociceptive effect of morphine in mice. Eur J Pharmacol 2000;399:161-4.
5. Joharchi K, Jorjani M. The role of nitric oxide in diabetes-induced changes of morphine tolerance in rats. Eur J Pharmacol 2007;570:66-71.
6. Kawamura N, Dyck PJ, Schmeichel AM, Engelstad JK, Low PA, Dyck PJ. Inflammatory mediators in diabetic and non-diabetic lumbosacral radiculoplexus neuropathy. Acta Neuropathol 2008;115:231-9.
7. Younger DS, Rosoklija G, Haya AP, Trojaborg W, Latoy N. Diabetic peripheral neuropathy: a clinico-pathologic and immunohistochemical analysis of sural nerve biopsies. Muscle Nerve 1997;20:520-1.
8. Tang Q, Adams JY, Penaranda C, Melli K, Piaggio E, Sgouroudis E, *et al*. Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. Immunity 2008;28:687–97.
9. Maigan AH, Clive H, Wasserfall MA, Atkinson TM. Central Role for Interleukin-2 in Type 1 Diabetes. Diabetes 2012;61:14-22.
10. Piemonti L, Maffi P, Monti L, Lampasona V, Perseghin G, Magistretti P, *et al*. Beta cell function during rapamycin monotherapy in long-term type 1 diabetes. Diabetologia 2011;54:433–9.
11. Kamei J, Kawashima N, Suzuki T, Misawa M, Kasuya Y. The effect of cyclosporin on morphine-induced antinociception in diabetic mice. Neurosci Lett 1993;158:213-6.
12. Homayoun H, Khavandgar S, Namiranian K, Dehpour AR. The effect of cyclosporin a on morphine tolerance and dependence: involvement of L-arginine/nitric oxide pathway. Eur J Pharmacol 2002;452:67-75.
13. Förstermann U, Closs EI, Pollock JS, Nakane M, Schwarz P, Gath I. Nitric oxide synthase isozymes: Characterization, purification, molecular cloning, and functions. Hypertens 1994;23:1121-31.
14. Gilhotra N, Sharma A, Singh M, Dhingra D. Involvement of p38MAPKinase in attenuation of antinociceptive effect of morphine. Indian J Exp Biol 2007;45:654-6.
15. Abdel-Zaher AO, Hamdy MM, Aly SA, Abdel-Hady RH, Abdel-Rahman S. Attenuation of morphine tolerance and dependence by aminoguanidine in mice. Eur J Pharmacol 2006;540:60-6.
16. Ahmet H. Animal models of peripheral neuropathies. Neurotherapeutics 2012;9:262–9.
17. Demiot C, Tartas M, Fromy B, Abraham P, Saumet JL, Sigaudo-Roussel D. Aldose reductase pathway inhibition improved vascular and C-fiber functions, allowing for pressure-induced vasodilation restoration during severe diabetic neuropathy. Diabetes. 2006;55:1478-83.
18. O'Brien PD, Sakowski SA, Feldman EL. Mouse models of diabetic neuropathy. ILARJ. 2014;54:259-72.
19. Trinder P. Estimation of plasma glucose by glucose oxidase method. Ann Clin Biochem 1969;6:24.
20. D' Amour WL, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther 1941;72:74-9.
21. Haley TJ, McCormick WG. Pharmacological effect produced by intracerebroventricular injection of drugs in mice. Br J Pharmacol 1957;12:12-5.
22. West WH, Tauer KW, Yannelli JR, Marshall GD, Orr DW, Thurman GB, *et al*. Constant-infusion recombinant interleukin-2 in adoptive immunotherapy of advanced cancer. N Engl J Med 1987;316:898-905.
23. Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, *et al*. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 of high dose interleukin-2 alone. N Eng J Med 1987;316:889-97.
24. Tang Q, Adams JY, Penaranda C, Melli K, Piaggio E, Sgouroudis E, *et al*. Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. Immunity 2008;28:687–97.
25. Ohkawa F, Ikeda U, Kane T, Kawasaki K, Shimada K. Inflammatory cytokines and rat vascular tone. Clin Exp Pharmacol Physiol 1995;12:S169-71.
26. Won SJ, Huang WT, Lai YS, Lin MT. Staphylococcal enterotoxin A acts through nitric oxide synthase mechanism in human peripheral blood mononuclear cells to stimulate the synthesis of pyrogenic cytokines. Infact Immune 2000;68:2003-8.