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Antinociceptive action of FK506 in mice

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Immunophilins are abundantly present in the brain as compared to the immune system. Immunophilin-binding agents like FK506 are known to inactivate neuronal nitric oxide synthase (nNOS) by inhibiting calcineurin and decrease the production of nitric oxide. Nitric oxide is involved in the mediation of nociception at the spinal level. In the present study, the effect of FK506 on the tail flick response in mice and the possible involvement of NO-L-arginine pathway in this paradigm was evaluated. FK506 (0.5, 1 and 3 mg/kg, ip) produced a significant antinociception in the tail flick test. Nitric oxide synthase (NOS) inhibitor L-NAME significantly and dose dependently (10-40 mg/kg, ip) potentiated the FK506 (0.5 mg/kg)-induced antinociception. On the other hand, NOS substrate L-arginine (100, 200 and 400 mg/kg) inhibited the FK506-induced antinociception in a dose-dependent manner. Concomitant administration of L-NAME (20 and 40 mg/kg) with L-arginine (200 mg/kg) blocked the inhibition exerted by L-arginine on the FK506-induced antinociception. Thus, it was concluded that NO- L-arginine pathway may be involved in the FK506-induced antinociception in tail flick test.

Keywords: Antinociception, FK506, Mice

Nitric oxide (NO) is an important mediator of various physiological functions in the central and peripheral nervous systems[1]. It has been demonstrated to have pro-nociceptive activity in the spinal cord[2,3]. NO donors, like L-arginine enhanced the release of substance P and calcineurin gene-related peptide which are involved in nociception in the spinal cord[4]. On the other hand, nitric oxide synthase (NOS) inhibitors like L-NAME abolished the antinociceptive response in tail flick assays[5].

FK506 (tacrolimus), an immunosuppressant drug isolated from *Streptomyces tsukubaensis*, is used worldwide to prevent allograft rejection and with relatively diminished side effects, notably nephrotoxicity[6]. FK506 has shown neuroprotective effect against 6-OHDA-induced toxicity in mice[7] and also reduced the increased level of TNF-alpha level in the nigrostriatal dopaminergic region injured by 6-OHDA in rats[8]. It has been shown that immunophilins, protein receptors for immunosuppressant drugs like cyclosporine and FK506, are widely distributed in the brain as compared to immune system[9]. One of the important roles of immunophilins in the neural functions envisaged is the regulation of NO-induced neurotoxicity[10]. FK506 by inhibiting calcineurin[6] indirectly reduces the catalytic activity of nNOS thereby decreasing NO production. Thus it provides neuroprotection like other NOS inhibitors[9].

As stated above NOS plays an important role in both nociception and antinociception. In the present study, the nNOS inactivating property of FK506 was utilized to examine its role in the modulation of tail flick response. Further, the possible involvement of L-arginine NO pathway in this response was evaluated.

Materials and Methods

Animals — Male albino mice (Laka strain) of either sex (20-30g), bred in Central Animal House facility of the Panjab University were used. The animals were housed under standard laboratory conditions, maintained on a natural light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the test. All experiments were carried out between 0900 and 1700 hrs. The experimental protocols were approved by the Institutional Ethics Committee and conducted according to the Indian National Science Academy guidelines for the use and care of experimental animals. Each mouse was used only once and each treatment group consisted of 6 animals.

Tail-flick test (radiant heat-induced nociception) — The analgesic response was determined by measuring the tail-flick latency to radiant heat [11]. The baseline latency to the tail withdrawal from the radiant heat

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source (3-5 s) was established. A cut- off time of 10 s was used to prevent any injury to the tail. Three trials were recorded for each animal to calculate the mean basal latency. The increase in latency (nociceptive threshold) was recorded at, 30, 60, 120, 180, 240 and 300 min after drug administration. The observer was unaware of the specific treatment schedule while performing the experiments.

Drugs—The following drugs were used: FK506 (supplied by Archer Chemicals, Mumbai, India), L-arginine (Himedia, Mumbai, India) and L-NAME (Sigma, St. Louis, USA). FK506 was suspended in 0.5% carboxymethyl cellulose. L-arginine, and L-NAME were dissolved in saline. All drugs were administered intraperitoneally (ip). Control animals received corresponding amount of vehicle (0.5% carboxymethyl cellulose) or saline.

Treatment schedule—In the first group of animals, the effect of acute administration of FK506 (0.5-3 mg/kg) on tail flick response was assessed 30, 60 120, 240 and 300 min after the drug administration. The second group of mice received L-NAME (10, 20 and 40 mg/kg) 30 min before the administration of FK506 (0.5 mg/kg). Third group of mice received L-arginine (50, 100, 200 and 400 mg/kg) 30 min before the administration of FK506 (3 mg/kg). Two other groups received L-NAME (20 and 40 mg/kg) concomitantly with L-arginine 30 min before the administration FK506 (3 mg/kg).

Statistical analysis—Results were expressed as mean ± SE. The significance in the difference in responses to various treatment groups in comparison to the control was determined by one-way analysis of variance (ANOVA) followed by Dunnet's test. $P < 0.05$ was considered statistically significant.

Results

Effect of FK506 on tail flick latency and its modification by L-NAME and L-arginine—Administration of FK506 (0.5-3mg/kg) produced analgesia as it increased the tail flick latency with the maximum effect being observed with 3 mg/kg after 2 hr of drug administration ($P<0.05$) (Fig. 1).

A lower dose (20 mg/kg) of L-NAME, a NOS inhibitor did not produce any significant change in tail flick latency, while a higher dose (40 mg/kg) increased the latency. Pretreatment with L-NAME significantly potentiated the antinociceptive effect of FK506 (0.5 mg/kg) (Fig. 2). L-arginine, a NO donor and a NOS substrate showed a significant nociceptive effect *per se* in doses studied (100, 200 and 400 mg/kg). There was a significant reduction in tail flick latency at 200 and 400 mg/kg doses. Pretreatment with L-arginine also reversed the antinociceptive effect of FK506 (3 mg/kg). At a dose of 200 mg/kg, L-arginine completely inhibited the FK506-induced antinociception, while the higher dose of L-arginine (400 mg/kg) produced pronociception in FK506-treated animals (Fig. 3). Concomitant administration of L-NAME (20 and 40 mg/kg) with L-arginine (200 mg/kg) reversed the L-arginine-induced inhibition of FK506 (3 mg/kg) antinociception (Fig. 4).

Discussion

In acute administration of FK506 produced antinociceptive effect in the tail-flick assay. L-arginine, a NOS substrate completely and dose dependently reversed the antinociceptive effect of FK506, L-NAME, a NOS inhibitor on the other hand potentiated the effect of FK506.

Immunophilins are the receptor proteins for the major immunosuppressant drugs such as cyclosporine A, FK506 and rapamycin[12]. The majority of the research on immunophilins and their ligands has focused on the cells of the immune system, especially lymphocytes, but the findings that immunophilins are abundantly present in the nervous system have lead to the investigations on the role of immunophilins in the neural functions. These include regulation of NO toxicity, neurotransmitter release, intercellular calcium flux, as well as neurotrophic influences with possible therapeutic potentials[13, 14].

In the nervous system, FK506 by binding to calcineurin, increases the phosphorylated levels of nNOS causing a reduction in the catalytic levels of nNOS; which may contribute to the neuroprotective effect as shown by other NOS inhibitors[9]. The doses employed in the present study have been reported to be insufficient to prevent rejection of neuronal transplants in the rat[15] and hence appears to lack immunosuppressive effects. However, FK506 has been shown to exhibit neuroprotection even in very small doses (% of the immunosuppressive dose) in animal models of neuroprotection[16].

Nociception mechanism(s) known are well reported to involve L-arginine NO pathway. The findings that L-NAME produces antinociception at high doses and L-arginine produces nociception are in accordance with previous reports [17]. The inhibitors of NOS can produce antinociception at high doses and NOS substrates may have *per se* antinociceptive effect, respectively.

Interestingly, in the present study L-NAME dose dependently potentiated the FK506-induced antinociception. A reduction in the activity of the NOS in the nervous system could be the probable mechanism of FK506-induced antinociception. The reversal of FK506-induced antinociception by L-arginine or facilitation further supports this hypothesis. FK506-induced antinociception was abolished by lower dose of L-arginine (100 mg/kg); at this dose it did not produce any nociception *per se*. Thus, the increased NO synthesis due to L-arginine may have compensated for the decreased NOS activity due to FK506.

Concomitant administration of L-NAME with L-arginine blocked the inhibitory effect of L-arginine on FK506-induced antinociception in a dose-dependent

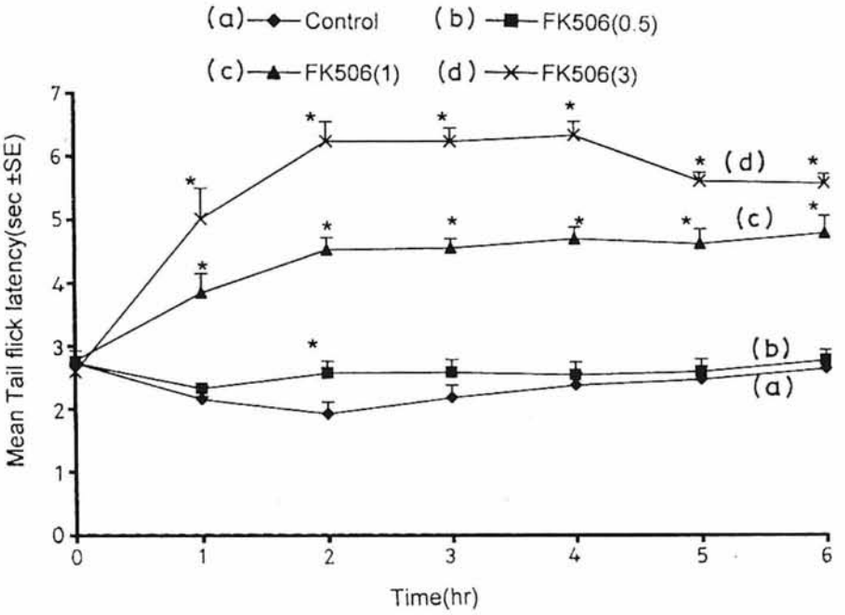

Fig. 1 — Time and dose-dependent antinociception induced by acute administration of FK506 (0.5-3 mg/kg, ip). Values are mean ± SE. $*P < 0.05$ as compared to the vehicle treated group (n = 5-8).

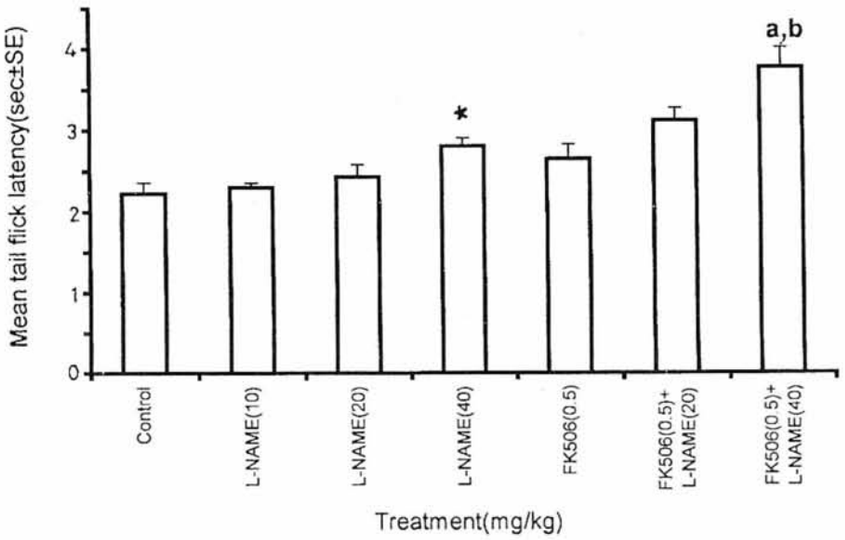

Fig. 2 — Effect of L-NAME (10, 20 and 40 mg/kg, ip) on the antinociceptive action of FK506 (0.5 mg/kg, ip). L-NAME was administered 30 min and 150 min before the tail flick test. Values are mean ± SE. *P* values:* < 0.05 as compared to the vehicle treated group . [a]< 0.05 as compared to the FK506 (0.5 mg/kg) treated group (n= 5-8); . [b]<0.05 as compared to the L-NAME (40 mg/kg) treated group

manner. This further confirms the hypothesis that FK506 has a modulatory effect on NOS and thus NO synthesis. This is in accordance with the fact that L-NAME in a dose dependent manner reverses the effect of L-arginine in morphine analgesia[18].

FK506 and other non-immunosuppressive derivates are in clinical trials for their use in CNS disorders such as Parkinson and Alzheimer's diseases but there are no reports till date on the clinical use of FK506 as an antinociceptive agent. Further studies are required to establish the use of FK506 and other non-immunosuppressive derivates as analgesics clinically. In conclusion FK506 produces an antinociceptive response in a dose dependent manner on acute

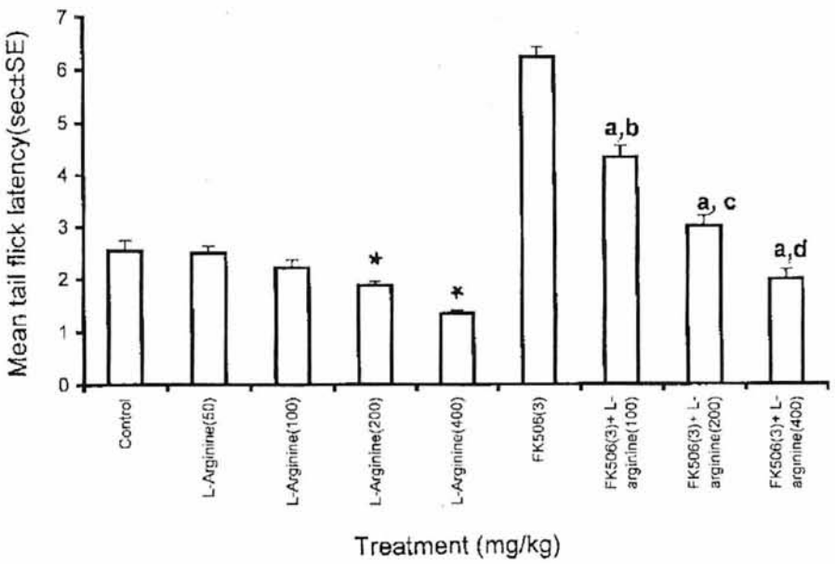

Fig. 3 — Effect of L-arginine (50,100, 200 and 400 mg/kg, ip) on the antinociceptive action of FK506 (3 mg/kg, ip). L-Arginine was administered for 30 min and 150 min before the tail flick test. Values are mean ±SE. *P* values: *< 0.05 as compared to the vehicle treated group . [a]< 0.05 as compared to the FK506 (3 mg/kg) treated group (n = 5-8). [b,c,d]< 0.05 as compared to the L-arginine (100,200,400 mg /kg) respectively treated group

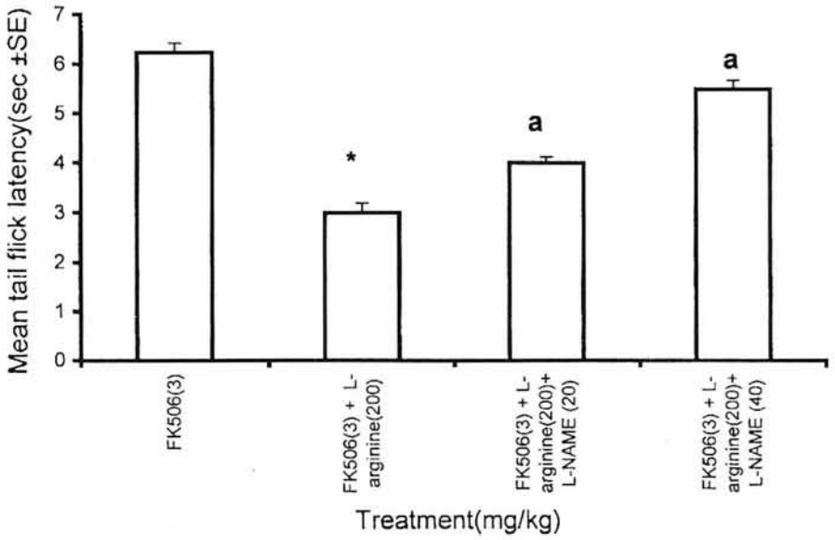

Fig. 4 — Effect of concomitant administration of L-NAME (10, 20 and 40 mg/kg) with L-arginine (200 mg/kg, ip) on the antinociceptive action of FK506 (3 mg/kg, ip). L-NAME and L-arginine was administered 30 min and 150 min before the tail flick test. Vales are mean ± SE. *P* values: *< 0.05 as compared to the FK506 treated group; [a]< 0.05 as compared to the FK506 (3 mg/kg)+ L-arginine(200 mg/kg) treated group (n = 5-8)

systemic administration and the possible involvement of NO L-arginine pathway in its antinociception actions is suggested.

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