Nimodipine potentiates the analgesic effect of morphine in the rat hot-plate test: Implications in the treatment of pain

INTRODUCTION

Opioids like morphine produce side effects ranging from nausea and vomiting, pruritus, oversedation, dizziness and urinary retention to respiratory depression.[1] Particularly, on chronic administration, it leads to development of tolerance.[2] Combining opioids with certain other drugs (adjuvant analgesics) like ketamine, which is an N-methyl-D-aspartate (NMDA) receptor antagonist, not only increases the analgesia, but also reduces the dose of opioids.[3] Previous research done in our laboratory and outside suggests that nimodipine, an L-type calcium channel blocker (L-CCBs), could be one such adjuvant drug.[4] Though originally used as an antihypertensive agent, its current use is restricted to the treatment of acute subarachnoid haemorrhage.[5] L-type calcium channels have been reported to mediate the major part of membrane calcium currents in the small-sized dorsal root ganglion neurons.[6] These neurons mediate the transmission of pain from the peripheral body parts to the central nervous system.

Recently, we reported that nimodipine, when co-administered with morphine had a greater therapeutic efficacy than either nifedipine or verapamil or diltiazem in the relief of pain in experimental animals.[4,7] In the present study, the analgesic effect of morphine/nimodipine or both was tested by the hot-plate nociceptive assay under experimental conditions that were different from that used in earlier works.[8] The results show that nimodipine could be co-administered with morphine for treating acute exacerbations of chronic pain as in the case of breakthrough pain. However, long-term treatment may not be useful. Further, low doses of nimodipine did not significantly interfere with the contraction of skeletal muscles as observed by the Rotarod test. The latter evaluates muscle strength and coordination. Muscle weakness could be an important side effect of L-CCB therapy as skeletal muscles also express an isoform of L-type channels.[9]

METHODS

Experimental animals and nociceptive assay

In the present work, analgesia was evaluated by the hot-plate apparatus (from Stoelting USA). Distinct groups of Wistar rats (weighing 175-225 g) received physiological saline (Group-I; n=6), morphine sulphate I.P. subcutaneously (20 mg/kg twice daily for 7 days followed by 30 mg/kg twice daily for another 7 days; Group-II; n=6), nimodipine (2 mg/kg once daily through intraperitoneal route; Group-III; n=6) or morphine (as in Group-II) with nimodipine (as in Group-III; nimodipine was administered 20 minutes before the morning dose of morphine) (Group-IV; n=6). The specific doses of nimodipine and morphine were selected based upon both toxicity studies conducted in the laboratory as well as previous literature on this topic.[4,7] The routes of administration of morphine (subcutaneous) and nimodipine (intraperitoneal) were different. This was due to the fact that intraperitoneal administration leads to quicker absorption into the blood as compared to subcutaneous administration. Thus, peak analgesic effect of morphine would coincide with high blood level of nimodipine. Morphine was purchased as morphine sulphate I.P. in ampoules (15 mg/ml) while nimodipine was from Sigma USA. Nimodipine was dissolved in a vehicle consisting of physiological saline, polyethylene glycol and absolute alcohol (2:2:1) under dim light.

The animals were maintained under 12 hours: 12 hours light and dark cycles and food and water

provided ad libitum. Prior permission for animal experimentation was obtained from the Institutional Animal Ethics Committee of AIIMS. Hot-plate latency period to hind paw licking or jumping was recorded by an observer blind to the drugs administered to the animals [Figure 1]. The time period of testing was 40 minutes after saline/morphine administration in Groups I-II. In group III (nimodipine only treated group), it was after 60 minutes. Finally, in Group IV, it was 40 minutes after morphine administration. It has been shown previously that maximum analgesic effect of morphine is achieved after 40 minutes of administration (also personal observation).[4] The plate temperature was maintained at 54 to 55°C. Cut-off time was set at 45 seconds, following which the animal was removed from the hot-plate to prevent tissue damage. The latency period was evaluated before starting the experiment and at the end of days 1, 2, 6, 10 and 14 of drug treatment.

Rotarod testing

The rats (n=30; Five groups of six rats each) were trained on the rotating rotarod apparatus (from Stoelting, USA) for 2 days at eight rotations per minute (r.p.m.).[10] The cut-off time was 300 seconds (s). On the third day, nimodipine (1/2/5 mg/kg), saline or vehicle (for nimodipine) was administered i.p. in different groups of rats. They were placed on the rotarod apparatus and the latency of fall was recorded after 60 minutes of drug administration. During this testing session, the Rotarod accelerated from 4 to 40 r.p.m. in 300 seconds. Lower values represent earlier fall and thus poor muscle strength.

Statistical analysis

Statistical analysis was done by ANOVA followed by Bonferroni multiple comparison test using the GraphPad Prism software (San Diego, USA). $P<0.05$ was considered significant.

RESULTS

Nociceptive assay

The analgesic effect of morphine produced an analgesic response, which started decreasing by day 2 and reached close to baseline by day 10, indicating the development of tolerance [Figure 2]. Compared to physiological saline, significant increase of analgesia was noted till day 2 for the morphine-treated group. Nimodipine co-administration increased the analgesic effect of morphine between days 2 to 6. However, nimodipine given alone did not produce any antinociception. Also, long-term treatment with morphine + nimodipine did not make a difference as evident from the hot-plate readings on days 10 and 14.

Figure 1: The hot-plate apparatus from Stoelting, USA used for testing pain response in the form of licking of hind paw or jumping. The cut-off time was 45 seconds

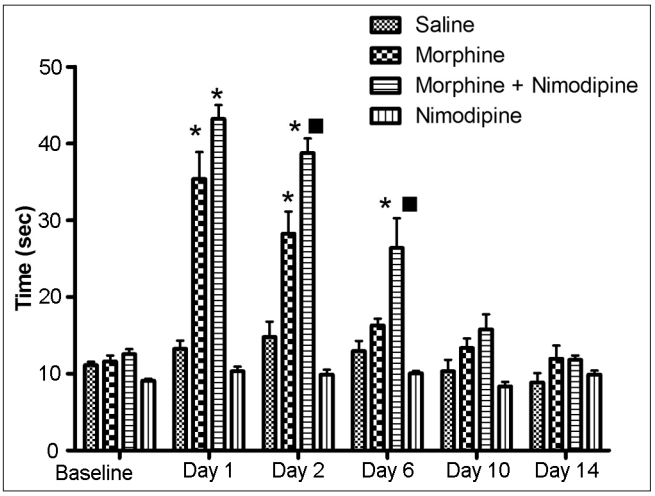

Figure 2: Hot-plate latency period in second(s) on different days of drug treatment. Higher latency period indicates greater analgesia. Morphine produced an analgesic effect which was significantly higher than saline on days 1 and 2 (*). Morphine with nimodipine group showed higher analgesia than saline on days 1, 2 and 6 (*). It also exhibited higher analgesia than morphine on days 2 and 6 (▪). Nimodipine alone did not have any analgesic effect. The values represent mean ± standard error of mean (s.e.m). $P<0.05$ was considered statistically significant

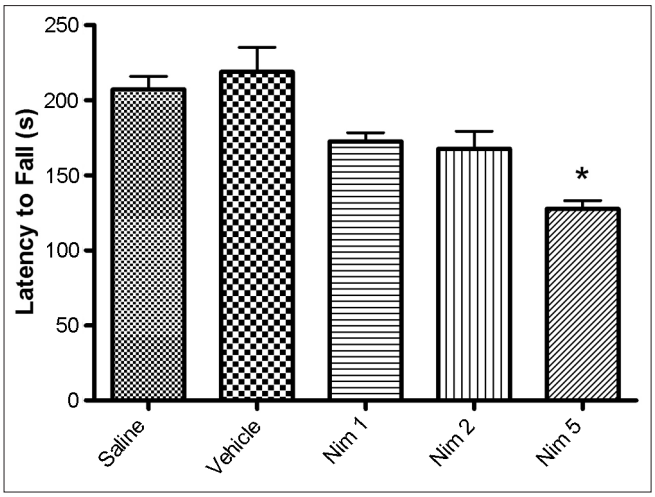

Figure 3: Latency of fall in seconds (s) during the Rotarod test. Nimodipine significantly decreased the latency of fall in comparison to saline at a dose of 5 mg/kg. Values are mean ± s.e.m. $P < 0.05$ was considered statistically significant

Rotarod testing

Compared to saline, administration of vehicle did not significantly affect the latency of falling [Figure 3]. Nimodipine (1 or 2 mg/kg) produced a non-significant reduction, though at the higher dose of 5 mg/kg, there was significant reduction in the latency to fall.

DISCUSSION

The result of the present study shows that nimodipine, an L-CCB, which did not have an analgesic action by itself, increased (potentiated) the analgesic effect of morphine. This is similar to our earlier findings in the tail-flick test and depicts synergism between these drugs.[4] However, there are certain differences between the earlier and the current study. The potentiation was noted in the later part of the observation period (day 12) in the earlier study which is in contrast to the current study where it was noted between days 2 to 6. In both cases, the total period of observation was 14 days. The difference can be correlated with the fact that the tail-flick response is a spinal reflex in comparison to the hot-plate test which is organised at the supraspinal level and thus is more representative of pain in human beings.[8] In both situations, the higher antinociceptive effect might be due to delay in the development of tolerance.

The mechanism responsible for the potentiation could be due to additional closure of L-type voltage-dependent calcium channels by nimodipine in neurons concerned with transmission of pain. This is besides closure of N- and P/Q-type voltage-dependent calcium channels by morphine in the presynaptic nerve terminals.[4] Others have also noted this facilitatory effect of L-CCBs on morphine-induced analgesia on chronic administration.[11-13] Michaluk *et al*. (1998) observed that both nifedipine (5 mg/kg) and verapamil (10 mg/kg), though not nimodipine (5 mg/kg), could delay the development of tolerance to morphine (20 mg/kg) in the hot-plate test.[13] It is possible that different experimental conditions could account for this variability. For example, the days on which the antinociceptive effect was recorded were different between the present (0, 1, 2, 6, 10 and 14) and the earlier study (1, 4 and 8). Also, on day 1, we noted maximum antinociceptive effect for the morphine with nimodipine group, which reached the cut-off time period (45 seconds). In contrast, the study by Michaluk *et al*. (1998) reported lower values.[13] Importantly, nimodipine might be safer than other L-CCBs due to its cerebroselective action.[14] No obvious side effects were observed in this study. Regarding blood pressure, Michaluk *et al*. (1998) had reported slight but significant decrease of the diastolic pressure only toward the end of the observation period (14^{th} day).[11] The authors had used a higher dose of nimodipine (5 mg/kg) in comparison to the present study (2 mg/kg). Presumably, the lower dose would not have affected the blood pressure. The dose of nimodipine appears to be important as higher doses (≥5 mg/kg) might produce muscle weakness as demonstrated by the Rotarod test. In human beings, a dose of 2 mg/h i.v. is administered for treatment of subarachnoid haemorrhage.[15] Using the conversion factor, its dose in a 200 g rat would be 0.86 mg.[16] A higher dose (2 mg/kg) was used in the current study as the route of administration was different (intraperitoneal rather than intravenous).

The applicability of the present work could be in treating conditions like breakthrough pain. These are temporary exacerbations of otherwise well-controlled pain and has a high incidence of occurrence (40-86%).[17] Such patients, who are already on opioid therapy, could be administered nimodipine through oral/parenteral routes for short durations of time. As mentioned earlier, treating these conditions by increasing the dose of opioids would lead to higher incidence of side effects. However, nimodipine administration alone would be counterproductive, as reported earlier from our laboratory.[7]

In conclusion, the result of the present study suggests

that nimodipine could potentiate the analgesic effect of morphine for short time periods and thus could prove useful in the treatment of pain.

ACKNOWLEDGEMENTS

The work was supported by an extramural research grant from the Indian Council of Medical Research, N. Delhi.

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Quick Response Code:	Website: www.ijaweb.org
	DOI: 10.4103/0019-5049.84843