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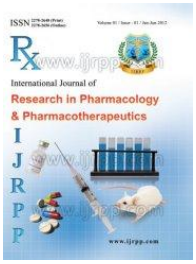

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Amlodipine Potentiates Antinociceptive Activity of Ketorolac and Tramadol – An Experimental Study

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ABSTRACT

OBJECTIVE

Dihydropyridines such as cilnidipine and amlodipine have been shown to block not only L-type but also N- type calcium channels. Ketorolac, a peripherally acting NSAID analgesic & tramadol, a centrally acting opioid analgesic are equipotent in treatment of mild to moderate acute pain. This study proposes to find any possible antinociceptive action of amlodipine and whether it potentiates analgesic activity of ketorolac and tramadol.

Methods

Adult healthy Wistar albino rats were grouped into 16 groups. The experiment was carried out using tail- flick method by analgesiometer. Different doses of amlodipine (2.5, 3, 3.5 mg/kg), ketorolac (15, 30, 45 mg/kg) and tramadol (10, 25, 50 mg/kg) and were administered intraperitoneally to select nonanalgesic doses. Different doses of amlodipine was combined with nonanalgesic doses of ketorolac & tramadol to study antinociceptive effect of combinations.

Results

Ketorolac and tramadol showed dose dependent antinociception which peaked at 2 hours. Amlodipine alone showed antinociceptive action at a dose of 3.5 mg/kg after 6 hour of administration. Higher doses of amlodipine (3, 3.5 mg/kg) in combination with nonanalgesic dose of tramadol produced significant antinociception. But Amlodipine at all dose potentiated the antinociceptive action of subanalgesic dose of ketorolac.

Discussion and conclusion

It can be concluded that amlodipine at high dose produced antinociceptive action. Combination of amlodipine with ketorolac and tramadol produced significant enhancement of antinociceptive activity of both tramadol & ketorolac in dose dependent manner.

Keywords: N-type calcium channel, Amlodipine, Analgesia

INTRODUCTION

Ketorolac is one of the most potent NSAID having similar analgesic efficacy as morphine or pethidine in relieving post-operative and acute pain but with very few side effects[1]. Ketorolac has a synergistic effect with morphine and fentanyl thus reduces the need for opiates and side effects of both.[2,3] Ketorolac is effective as analgesic, antiinflammatory and

antipyretic.[4] In rats its analgesic activity is 300-500 times that of aspirin.[5]

Tramadol hydrochloride, a centrally acting analgesic, is a synthetic codeine analogue. It acts as μ- opioid receptor agonist and activates monoaminergic spinal inhibition of pain by inhibiting reuptake of serotonin and norepinephrine. It has lower side effects in comparison to typical opioid agents[6]. Tramadol is as effective as morphine or pethidine in treatment of mild to moderate pain, but is less effective in severe or chronic pain.

Amlodipine, a dihydropyridine (DHP) type calcium channel blocker used in hypertension and angina, shows benefit by acting on L- type calcium channels. Further studies revealed that amlodipine[7]. and cilnidipine.[8] block N- type calcium channel also. This finding indicates that DHPs are no longer considered L-type specific blocker and suggest that some DHPs may block other subtypes of calcium channels (N, P/Q, R types). It has been found that blockade of voltage gated calcium channel results in antinociception. The non- L- type calcium channels are diversely distributed in peripheral and central nervous cells.[9] N- Type calcium channels are found primarily at presynaptic terminals and are involved in neurotransmission.[10] Strong depolarisation by an action potential causes these channels to open and allow influx of calcium, initiating vesicle fusion and release of stored neurotransmitter. N and L- type calcium channels can be blocked by endogenous chemical transmitters and drugs to prevent nociceptive signaling.[9]

However pharmacological profiles of the effects of DHP on each calcium channel subtype are not understood well enough to be used with confidence in other situations. Thus we have proposed to study the antinociceptive action of amlodipine alone and when combined with subanalgesic doses of tramadol and ketorolac separately.

MATERIALS AND METHODS

The study was conducted on healthy albino Wistar rats (180-250 gm) of either sex. They were housed in the departmental animal house, in cages, in temperature regulated rooms with air cooling and 12 hour light and dark cycle, under standard laboratory conditions and fed with standard animal feed and were given water *ad libitum*. They were allowed to acclimatize to the laboratory conditions for a period of one week. The experimental protocol was approved by the institutional animal ethics committee. The animals were divided into sixteen groups each containing six, as shown in Table 1 and were administered with standard analgesics i.e. ketorolac, tramadol, the test drug amlodipine and each of the standard analgesic drugs were also combined with amlodipine to observe the intervention.

Table 1: Groups of animals given varius drugs

Group of animals	Drug	Dose
1.	normal saline	5ml/kg
2.	ketorolac	15mg/kg
3.	ketorolac	30mg/kg
4.	ketorolac	45mg/kg
5.	tramadol	10mg/kg
6.	tramadol	25mg/kg
7.	tramadol	50mg/kg
8.	amlodipine	2.5mg/kg
9.	amlodipine	3mg/kg
10.	amlodipine	3.5mg/kg
11.	ketorolac + amlodipine	15mg/kg+2.5mg/kg
12.	ketorolac + amlodipine	15mg/kg+3mg/kg
13.	ketorolac + amlodipine	15mg/kg+3.5mg/kg
14.	tramadol + amlodipine	10mg/kg+2.5mg/kg
15.	tramadol + amlodipine	10mg/kg+3mg/kg
16.	tramadol + amlodipine	10mg/kg+3.5mg/kg

Drugs

Amlodipine besylate was obtained from Macleods Pharmaceuticals Ltd., Himachal Pradesh, India.
Ketorolac was obtained from Dr. Reddy's Laboratories Ltd., Hyderabad, India.

ketorolac tromethamine was obtained from Neon Laboratories Ltd., Maharashtra, India.

Instrument

Digital analgesiometer of INCO Ambala Company, India with digital current meter and current setter was used.

Determination of analgesic activity

Antinociceptive activity was measured by tail flick method using analgesiometer. Radiant heat from an electric source was used as a stimuli and the time required for the sudden flicking of the tail was considered as the 'reaction time' or 'the tail flick latency' (TFL). In order to prevent tissue injury due to repeated exposure to the heat stimulus, the 'cutoff time' was considered as twenty seconds.[11]

The baseline reaction time was obtained at the start of experiment (0 hrs) just before the drug administration. In case of the groups in which only control (saline), ketorolac and tramadol were given (group 1- 7), TFL test was repeated at 0.25, 0.5, 1, 1.5 and 2 hrs after the administration of the drugs as the time to reach peak effect of ketorolac and tramadol is 1 and 2 hours respectively. In case of the groups in which amlodipine alone (group 8-10) was given the test was repeated after 6, 6.5, 7, 7.5 and 8 hrs of administration of amlodipine as the peak effect of it was found at 8 hours.

In the groups receiving combined treatment, the two drugs were administered at different times. After observing baseline (0 hrs) reaction time at the start of experiment amlodipine was administered. Ketorolac (group 11-13) was given 5.5 hrs after the administration of amlodipine and the repeat test done at 6, 6.5, 7, 7.5 and 8 hrs from baseline. Similarly tramadol (group 14-16) was given 5 hrs after the administration of amlodipine and TFL test was repeated at 6, 6.5, 7, 7.5 and 8 hrs from baseline. All the drugs were administered intraperitoneally after preparing fresh solution.

Statistical Analysis

The results are expressed as mean ± S.E.M. One way ANOVA with post hoc test of significance was performed for comparison amongst different means. $P < 0.05$ was regarded as statistically significant.

RESULTS

Results of tail flick latency are showed in Table 2 and 3.

Table 2: Table showing tail flick latency (in seconds) in groups receiving normal saline, ketorolac, tramadol

Group	Drugs	Dose in mg/kg	TFL Duration(in seconds)					
			0 hr	0.25hr	0.5hr	1hr	1.5hr	2hr
1	normal saline	1.5ml	4.12±0.222	4.12±0.215	4.12±0.235	4.17±0.235	4.23±0.133	4.02±0.215
2	ketorolac	15	4.15±0.042	4.28±0.06	4.18±0.03	4.08±0.06	4.08±0.05	4.133±0.055
3	ketorolac	30	4.07±0.049	5.32*#±0.047	6.53*#±0.076	9.55*#±0.056	8.5*#±0.13	6.32*#±0.113
4	ketorolac	45	4.17±0.06	6.28*#±0.107	8.67*#±0.088	10.5*#±0.118	10.32*#±0.079	7.37*#±0.049
5	tramadol	10	4.15±0.076	4.45±0.76	4.31±0.113	4.17±0.108	3.93±0.105	3.75±0.076
6	tramadol	25	4.48±0.094	5.48*#±0.104	6.08*#±0.101	6.33*#±0.061	6.01*#±0.07	5.65*#±0.076
7	tramadol	50	4.13±0.105	10.43*#±0.27	11.67*#±0.182	12.3*#±0.159	12.42*#±0.132	12.03*#±0.114

(*,#P value < 0.05, statistically significant *compared to control, #compared to baseline)

Table 3: Tail flick latency (in seconds) in groups receiving normal saline, amlodipine alone and combinations of ketorolac and amlodipine, tramadol and amlodipine

Group	Drug	Dose	TFL Duration(in seconds)					
		mg/kg	0hr	6hr	6.5 hr	7hr	7.5hr	8hr
1	Normal saline	1.5 ml	4.12±0.222	4.12±0.215	4.12±0.235	4.17±0.235	4.23±0.133	4.02±0.215
8	Amlodipine	2.5	4.12±0.119	4.35±0.085	4.42±0.11	4.3±0.82	3.96±0.071	3.96±0.076
9	Amlodipine	3	3.67±0.105	3.93±0.092	4.02±0.13	3.73±0.13	3.55±0.126	3.45±0.099
10	Amlodipine	3.5	4±0.106	4.92*#±0.098	5.42*#±0.07	5.13*#±0.095	4.73*#±0.056	4.62*#±0.083
11	Ketorolac+ amlodipine	15+2.5	4.12±0.06	9.36*#±0.066	14.1*#±0.20	17.38*#±0.16	8.3*#±0.057	4.2±0.057
12	Ketorolac+ amlodipine	15+3	4.08±0.047	10.5*#±0.057	17.15*#±0.166	20*#±0	8.33*#±0.133	4.22±0.07
13	Ketorolac+ amlodipine	15+3.5	4.12±0.047	13.35*#±0.108	18.7*#±0.103	20*#±0	8.98*#±0.079	4.14±0.074
14	Tramadol+ amlodipine	10+2.5	4.35±0.117	4.5±0.1	4.37±0.172	4.3±0.096	4.03±0.114	3.78±0.117
15	Tramadol+ amlodipine	10+3	4.25±0.143	14.37*#±0.236	16.57*#±0.199	18.25*#±0.152	17.53*#±0.149	16.98*#±0.11
16	Tramadol+ amlodipine	10+3.5	4.3±0.123	17.93*#±0.326	20*#±0	20*#±0	20*#±0	18.6*#±0.232

(*,#P value < 0.05, statistically significant *compared to control, #compared to baseline)

Ketorolac in a dose of 15 mg/kg showed no significant effect on tail flick latency during the entire test period of two hours as compared to baseline and control. Ketorolac in doses of 30 mg/kg and 45 mg/ kg showed significant antinociceptive activity which started at 0.25 hr after drug administration and was maximum at 1 hr and and persisted for 1.5 hrs. ($p<0.01$), results of which are graphically shown in Figure 1.

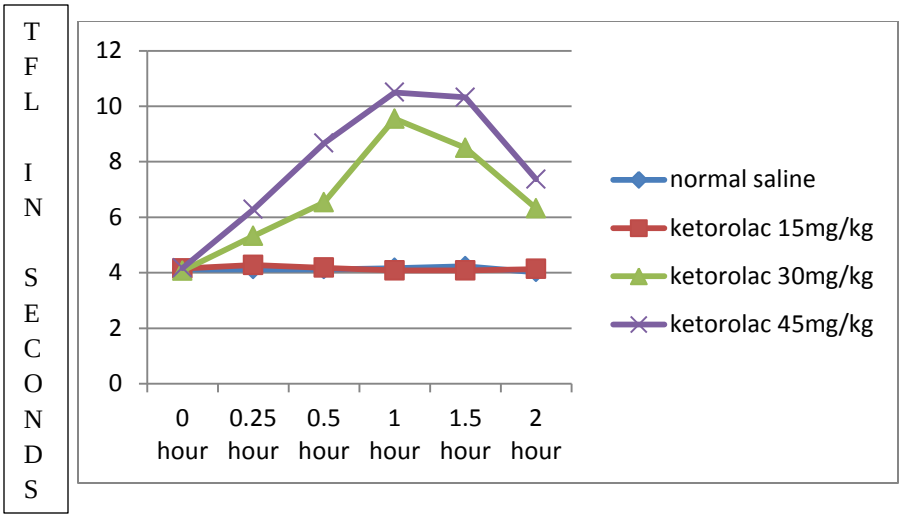

Figure 1: TFL produced by different doses of ketorolac compared with normal saline at different time interval

Tramadol in a dose of 10 mg/kg showed no significant effect on tail flick latency during the entire test period of two hours as compared to baseline and control. Tramadol in higher doses showed antinociceptive activity which started at 0.25 hr after drug administration and was maximum at 1 hr and and persisted for the entire test period of 2 hrs. (p<0.01). Results are depicted in Figure 2.

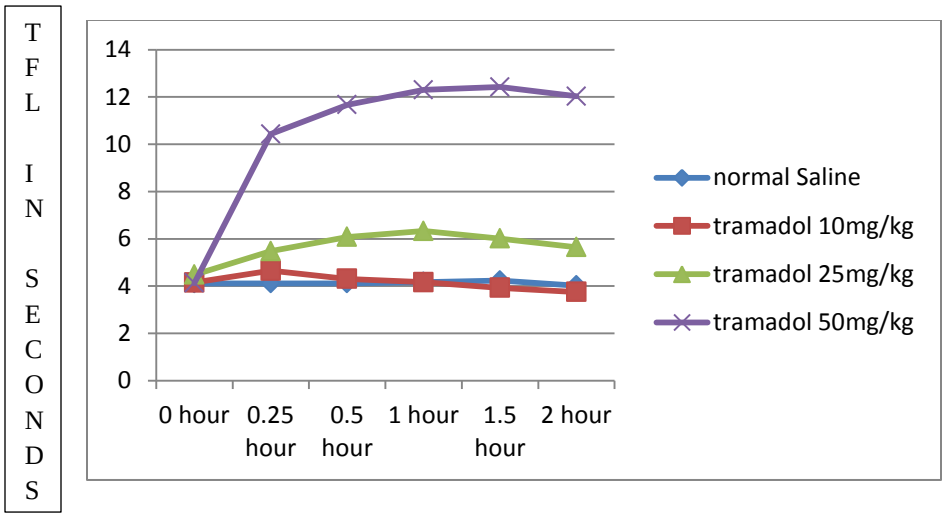

Figure 2: TFL produced by different doses of tramadol compared with normal saline at different time interval

Amlodipine in doses of 2.5 mg/kg and 3.0 mg/kg showed no significant change in the tail flick latency as compared to normal saline during the entire period of 6 hrs. However, the dose of 3.5 mg/kg showed antinociceptive effect from 6 hrs onwards with the peak effect at 6.5 hrs, which was significantly higher than control group and baseline value (p < 0.01). Results are graphically depicted in Figure 3.

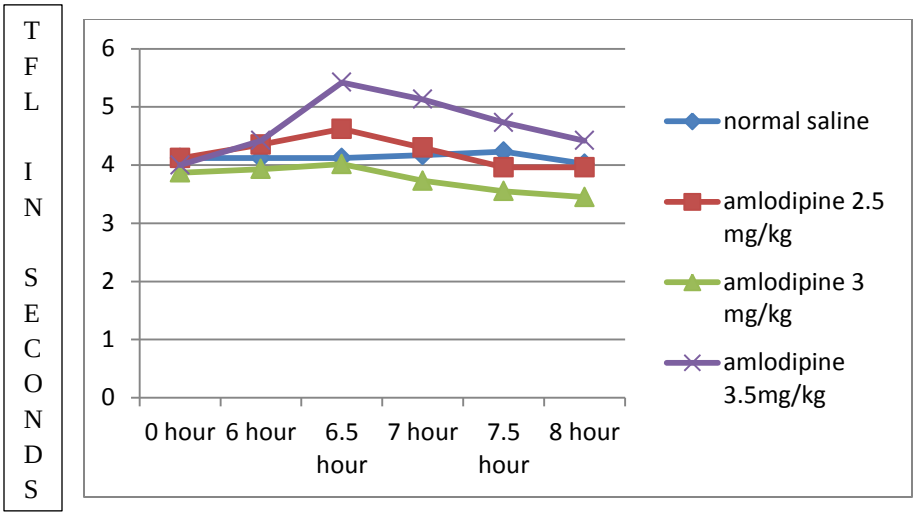

Figure 3: TFL produced by different doses of amlodipine compared with normal saline at different time interval

Combination of subanalgesic doses of Ketorolac (15 mg/kg) when combined with all doses of amlodipine showed significant increase in the tail flick latency, at 6 hrs onwards as compared to baseline and control with peak antinociceptive effect was observed at 7 hrs and decreased thereafter. The results are graphically depicted in Figure 4.

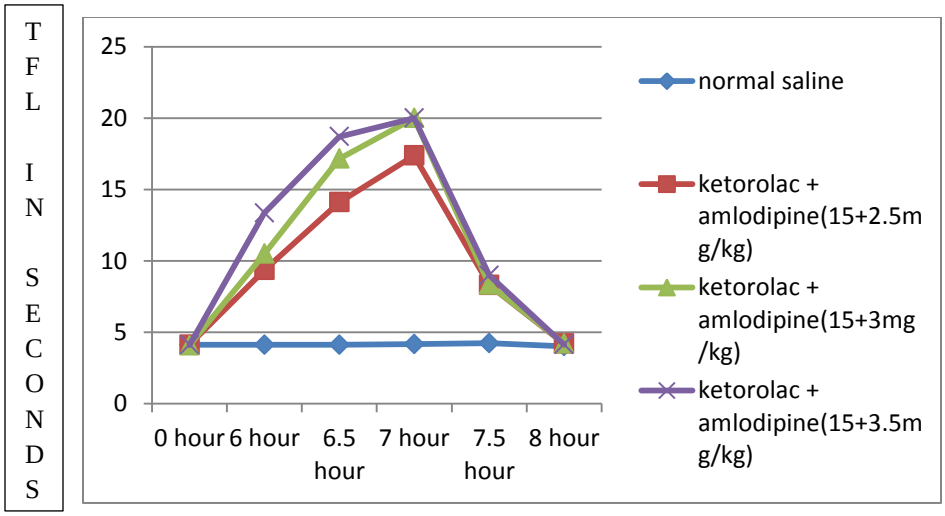

Figure 4: Comparison of TFL produced by combinations of ketorolac and different doses of amlodipine with normal saline

Combination of subanalgesic doses of tramadol (10 mg/kg) and low dose amlodipine (2.5 mg/kg) did not produce any significant increase in the tail flick latency during the entire period. Tramadol (10 mg/kg) when combined with higher doses of amlodipine showed a significant increase in the tail flick latency, at 6 hrs onwards as compared to baseline and control. The peak of the antinociceptive effect was observed at 6.5 and 7 hrs with highest and higher dose of amlodipine respectively and persisted significantly throughout the entire test duration, which is represented in Figure 5.

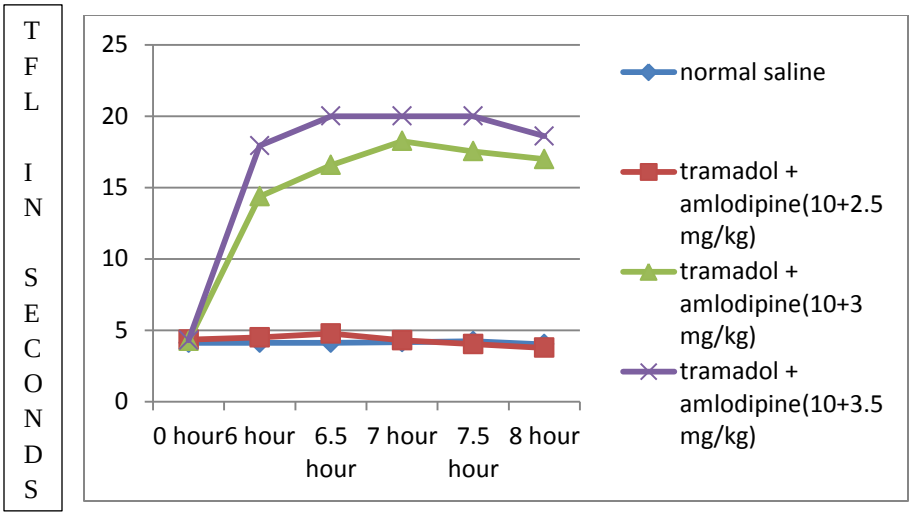

Figure 5: Comparison of TFL produced by combination of tramadol and different doses of amlodipine with normal saline

DISCUSSION AND CONCLUSION

Amlodipine increases tail flick latency at selectively higher dose (3.5mg/kg) when compared with control. This finding may be supported by previous literature in which intrathecal injection of amlodipine significantly shortened the licking in the second phase of formalin test.[10] Other N- type calcium channel blockers, cilnidipine, ω-conotoxin showed marked analgesic activity under similar experimental condition.[8] Blocking of calcium channel reults in attenuation of synaptic transmission of nociceptive neurons.[12] It has also been suggested that N –type calcium channel blocker may have analgesic activity when applied directly into subarachnoid space[12]. Thus the antinociceptive action may be attributed due to blockade of N- type calcium channels.

The combination of ketorolac and amlodipine reduced pain significantly than ketorolac or amlodipine alone. Our data is in conformity with one study which suggested that ketorolac may have a central modulatory effect on opioid pharmacology.[13] Other studies showed dose dependent synergistic antinociceptive action of N – type voltage dependent calcium channel blockers, ω-conotoxin and morphine.[14,15] The increased antinociceptive action of morphine may be possibly through a decrease in cellular calcium availability[10]. So the increased antinociceptive action of subanalgesic dose of ketorolac when combined with amlodipine may be due to N- type calcium channel blocking and decreased cellular calcium.

Tramadol does not produce analgesia at 10mg/kg dose when compared with control. It may occur probably due to the fact that tramadol is a μ- agonist and may poorly inhibit N- type calcium channels which is mainly ƙ – mediated.[6]

The increase in antinociceptive action of tramadol may be due to N- type calcium channel blocking action of amlodipine. This finding is in conformity to what has been found in Acetic acid Writhing test in mice, which reported that calcium channel blockers (diltiazem, verapamil, flunarazine, nicardipine, cinnarizine) produce antinociception and enhance antinociception of morphine.[16] In another study it was shown that intrathecal injection of N- type calcium channel blocker, ω- conotoxin with a Delta Opioid agonist (DADLE) was shown to be the most effective combination to produce antinociception in rat tail flick test.[17]

Amlodipine at high dose produced antinociceptive action. Amlodipine at low dose enhanced antinociceptive action of subanalgesic dose of ketorolac but not tramadol. At moderate and higher dose, amlodipine significantly increases the antinociceptive effect of both peripherally acting ketorolac and centrally acting tramadol. However these need to be further proved in other animal models and in clinical studies.

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