Acute analgesic effect of loperamide as compared to morphine after intrathecal administration in rat

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Loperamide, a mu opioid receptor agonist, which is commonly used as an antidiarrhoeal agent has been reported to possess analgesic activity after intrathecal administration. However, the exact analgesic profile, i.e., onset, duration and intensity of analgesia in relation to morphine is not fully known. In the present study, the acute analgesic effect of loperamide (5 μg) was compared with that of morphine (5 μg) and morphine + loperamide (5 μg of each) using the tail flick method after intrathecal administration. Naloxone (5 mg/kg) reversibility of the analgesic effect was also studied. The analgesic response of loperamide was significantly higher than morphine. Even after 22 hr, maximum possible effect was greater than 49%. Naloxone partially antagonized the analgesic effect of loperamide. This suggested that loperamide may be acting through blockade of Ca^{2+} channels besides activating mu opioid receptors. Loperamide may prove to be a better substitute for morphine as spinal analgesic.

Keywords: Analgesia, Calcium channel antagonist, Loperamide, Morphine, Mu-opioid receptor

Morphine produces analgesia by predominantly activating μ-opioid receptors. Loperamide is a synthetic opioid, which binds to μ-opioid receptors with higher affinity than morphine. It is a lipophilic, diphenylpiperidine derivative, which is used occasionally for the treatment of non-bacterial diarrhoeas. However, it produces potent antinociception when administered locally at the site of inflammation. This is due to its effect on peripheral μ-opioid receptors. Interestingly, co-administration of cereport along with loperamide allows the latter to penetrate the blood brain barrier and produce significant analgesic effect. It indicates that loperamide can produce an analgesic effect if directly instilled into the central nervous system or into the cerebrospinal fluid surrounding it.

A recent report has noted the analgesic effect of loperamide in 4-6 weeks mice after intrathecal administration in the formalin test. However, the analgesic effect of loperamide was not analysed with regard to its onset, duration or its magnitude, which is possible with tail flick method. Also, the analgesic effect was not compared with any other standard opioid analgesic drug like morphine. Hence, the analgesic profile of loperamide was studied in rat after standardization of the dose. Another reason was that earlier observations from our laboratory have shown that co-administration of morphine and nimodipine through the intrathecal route results in a higher analgesic effect than either of the drugs alone in both rats and mice. Incidentally, nimodipine is a L-type calcium channel blocker. Besides being an opioid, loperamide has also been shown to directly block multiple calcium channels. It appeared that loperamide could produce a higher analgesic effect than morphine alone. The present study has been undertaken to note whether loperamide may be a better substitute to morphine as spinal analgesic.

To evaluate the mechanism of analgesic effect of loperamide (opioidergic or non-opioidergic or both), naloxone an opioid antagonist, was systemically administered after 1 hr of administration of morphine and/or loperamide, when the maximum analgesia was achieved as evident by the tail flick response.

Materials and Methods

Adult male Wistar rats (n=56) weighing between 200-225 g were used. The rats were kept in cages with food and water ad libitum. The room temperature was maintained at about 25°C. Prior approval of Institutional Animal Ethics Committee (IAEC) was obtained.
The animals were divided into following 7 groups:
Group I (n=9): received 5 µg of loperamide, intrathecally (it)
Group II (n=13): received 5 µg of morphine, it
Group III (n=6): received 5 µg of loperamide + 5 µg of morphine, it
Group IV (n=6): received 10 µl of normal saline (0.9%), (it)
Group V (n=6): received 5 µg of loperamide (it) followed by naloxone (5 mg/kg) intraperitoneally (ip) after 1 hr
Group VI (n=8): received 25 µg of loperamide, (it), followed by naloxone (5 mg/kg), ip after 1 hr
Group VII (n=6): received 5 µg of morphine, (it), followed by naloxone (5 mg/kg), ip after 1 hr

Loperamide hydrochloride and naloxone was obtained from Sigma, USA. Morphine was obtained as morphine sulphate I.P. in ampules (15 mg/kg) from Government Pharmacy. Loperamide was dissolved in a solution of polyethylene glycol, normal saline and absolute alcohol in a ratio of 2:2:1. Loperamide and morphine were administered through single injection in Gr III. The solvent (for loperamide) was also separately injected (it) into a group of albino Wistar rats (n=3) to investigate whether it had any analgesic effect.

The total volume injected (it) was 10 µl. Loperamide was given at various doses (1, 5, 10, 25, 50 and 100 µg) initially. Higher doses (10–100 µg) frequently produced temporary muscular weakness of the hind limbs after intrathecal administration, which was absent at 5 µg. No analgesic effect was produced with 1 µg. A consistent analgesic effect without side effects was noted with 5 µg morphine, which was selected for the present study.

Muscular weakness was tested by the stepping and righting reflexes, which are tests for motor coordination and function after intrathecal administration. In the former, the rat is lifted by the tail so as to only slightly lift the hindlimbs off the surface when there is a extension of the hind limbs; when the tail is released, there is return of the hindlimbs to the surface. In the later test, the rat is rolled on to the dorsal aspect; the rat immediately returns to sternal recumbency.

For, injections (it), the lumbar region of the back was shaved by razor blade. Later, the skin was disinfected with povidone-iodine (5%) and injections (it) were given using sterile tuberculin syringes. This method was modified from an earlier described technique. The assessment of sensitivity to noxious thermal stimuli was done by the tail flick method using the tail flick apparatus (UGO Basile). Normally, rats flicked or move away their tails from the heat source between 2-4 sec (baseline latency). An upper cut off time of 10 sec was observed as further exposure to heat may damage the tail. Statistically 10 sec was regarded as equal to 100% response. The latency to tail flick was measured at 15 min, 30 min, 1 hr and thereafter every hour till 7 hr after (it) administration. For only loperamide (Gr I) and morphine (Gr II) groups, a repeat measurement was done at 22 hr. Maximum possible effect (MPE) was calculated as follows: (Post drug latency–baseline latency)/(cut off time–baseline latency) × 100.

Naloxone (5 mg/kg) was given (ip) 1 hr after 5µg loperamide administration in Gr V–VI and morphine administration in Gr VII. The total volume injected (ip) was 1 ml/kg of body weight. An additional group (Gr VI) with higher dose of loperamide (25 µg) was also administered naloxone. The tail flick latency was measured after 5 min and thereafter at every 5 min till 30 min. This is so because naloxone has a half-life of 1 hr.

Statistical evaluation was done using Kruskal-Wallis test (ANOVA). Individual groups were compared by Mann-Whitney U test. P-value<0.05 was considered significant.

Results
Administration of loperamide (Gr I) produced a tail flick response that exceeded the cut off limit of 10 sec (MPE=100%) by 15 min and remained at this level till 1 hr (Fig 1). Thereafter, the tail flick response slightly decreased but remained persistently above 9 sec (MPE >81%) till 7 hr. The analgesic response was maintained even 22 hr after the injection (MPE >49%). Co-administration of morphine + loperamide produced a tail flick response that exceeded cut off level by 15 min and which persisted at this level till 2 hr. Further on, it rapidly decreased to 6.8 see at 5 hr where it reached a plateau till about 7 hr (7 sec–MPE of 58%). The antinociceptive effect of morphine alone (Gr II) caused the tail flick response to exceed cut off level at 30 min and persist at this level till 1 hr. Thereafter, it rapidly declined to reach control reading at predrug state at 5 hr (9.3% of MPE) and persisted at about this level till 7 hr. Administration of normal saline did not produce significant analgesic response and values for tail flick test remained close to baseline till 3 hr.
Loperamide (Gr I) produced analgesia, which was significantly higher as compared to morphine (Gr II) between 2 - 7 hr. Even at 22 hr, the analgesic response of Gr I was significantly higher than Gr II. Analgesia in Gr I (loperamide) was significantly higher than Gr III (morphine + loperamide) at 5 and 6 hr. Morphine + loperamide produced significantly higher analgesic than morphine between 2-7 hr. Analgesic response of morphine was significantly higher than control (saline) up to 2 hr after administration.

Naloxone reversed the maximum analgesic response of morphine (Gr VII) to a significant extent as evident by the tail flick response (Fig. 2). It remained at this lower level till 30 min. The significance was determined by Student’s t test as we were comparing the same group at different time points. However, for loperamide, such a dramatic decrease in response was absent. In fact, there was fluctuation of the tail flick response. Significant fall in analgesic effect of morphine as compared to loperamide (5 μg) was observed between 1:15 to 1:30 hr. However, with a higher dose of loperamide (25 μg), in addition to the above mentioned time points (1:15 to 1:30 hr), significant decrease of analgesic effect was also noted at 1:05 hr. This indicated that the analgesic effect of loperamide was relatively resistant to naloxone.

Rats injected with solvent for loperamide (it) did not show any analgesic response. No motor deficits were noted except in one rat from Gr I lasting for 5 min. Specifically this rat showed defective stepping and righting reflexes.

Discussion

The present study showed that intrathecal administration of loperamide (Gr I) produced superior
analgesic response as compared to morphine (Gr II) when administered by the same route. The analgesic response persisted at a higher level till about 6 hr (MPE >81%) after loperamide administration as compared to 1 hr after morphine administration. 49.1% of the maximum possible analgesic effect (MPE) persisted in loperamide treated animals even after 22 hr. This is remarkable as it shows that analgesia due to loperamide is several fold more effective than morphine.

Interestingly, naloxone failed to reverse the analgesic effect of loperamide and morphine in a uniform manner. It appears that only a part of the analgesia produced by loperamide is mediated through its binding to μ-opioid receptors, indicating that other mechanisms may be involved. In this regard, loperamide has been shown to prevent calcium ion influx by blocking high-voltage-activated calcium channels in pyramidal neurons of hippocampus. This effect was not antagonized by naloxone, which indicates that it was not mediated through opioid receptors. In this regard, a recent study has shown that calcium influx into neurons of dorsal root ganglia was strongly inhibited by loperamide, which presumably was due to simultaneous blockade of L-, P/Q- and N-type calcium channels. It appears that blockade of the calcium channels could be the other mechanism responsible for the potent antinociceptive action of loperamide. Since calcium plays an important role in the release of neurotransmitters from pre-synaptic sites, blockade of calcium influx may decrease the excitability of neurons of the dorsal horn. This would also include neurons transmitting pain signals and hence the analgesic effect. Even the antidiarrhoeal action of loperamide may be partly due to its role in blocking calcium channels. Voltage-gated sodium channels is blocked by loperamide too. Moreover, it has been shown that loperamide can also bind to yet unidentified opioid receptors or to heterodimers of opioid receptors. All these could be responsible for the higher analgesic effect of loperamide.

Another very important observation in the present study was the decrease in analgesic effect of 5 μg each of loperamide + morphine as compared to 5 μg of loperamide alone. A possible explanation could be that morphine prevents binding of loperamide to μ-opioid receptors. Morphine being a drug with lower efficacy may not induce as much analgesia as loperamide. Thus, combining loperamide with any other μ receptor agonist would prove counter-productive.

Regarding side effects of loperamide, temporary paralysis of hind limbs in about 50% of experimental animals lasting for about 20-30 min with 25 μg of loperamide. However, with 5 μg of loperamide, only one rat in Gr I (6.7% of the total) and none in any other group which received loperamide showed a temporary weakness lasting for about 5 min. It is possible that this effect could be due to blockade of calcium channels in the motor neurons of the anterior horn of spinal cord.

In conclusion, the present study indicates that loperamide appears to be a very promising drug for producing spinal analgesia of long duration. However, future studies particularly neurotoxicity studies are needed to exclude any long-term damage to the spinal cord.

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References

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