Chronic spinal infusion of loperamide alleviates postsurgical pain in rats

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Plantar incision in rat generates spontaneous pain behaviour. The opioid drug, morphine used to treat postsurgical pain produces tolerance after long-term administration. Loperamide, a potent mu-opioid agonist, has documented analgesic action in various pain conditions. However, loperamide analgesia and associated tolerance following continuous spinal administration in postsurgical pain has not been reported. Chronic spinal infusion of drugs was achieved using intrathecal catheters connected to osmotic minipump. Coinciding with the onset of spinal infusion of loperamide or morphine, rats were subjected to plantar incision. Pain-related behaviour was assessed by Hargreaves apparatus (thermal hyperalgesia) and von Frey filaments (mechanical allodynia). Morphine and loperamide (0.5, 1 and 2 µL/h) induced analgesia was observed until 7th day post-plantar incision in Sprague-Dawley rats. Morphine and loperamide produced dose-dependent analgesia. Loperamide, in the highest dose, produced analgesia till 7th day. However, the highest dose of morphine produced inhibition of thermal hyperalgesia till 5th day and mechanical allodynia only till 3rd day post-plantar incision. Morphine and loperamide produced analgesia in postsurgical pain, which may be mediated through different mechanisms. Longer duration of analgesia with loperamide could probably be due sustained blockade of calcium channels.

Keywords: Hargreaves test, Intrathecal catheterization, Loperamide, Morphine, Osmotic minipump, Plantar incision, von Frey test

Patients with cancer pain, including those with a neuropathic component benefit from treatment with chronic intrathecal morphine. Also, morphine forms the main-stay of postoperative analgesia. However, sustained administration of morphine leads to progressive loss of analgesia and shifts the dose-response curve to the right indicating tolerance. Additionally, concerns regarding addiction and the potential for abuse limit its use as analgesic.

Loperamide is a mu-opioid receptor (MOR) preferred agonist that does not cross the blood–brain barrier1. It produces analgesia after peripheral2-4 as well as neuraxial (intracisternal/intrathecal) administration5-9. Further, the analgesic response of loperamide has been noted to be both earlier in onset and of longer duration than morphine8,9. The systemic and local administration of loperamide reverse behavioural hypersensitivity to noxious mechanical and thermal stimuli in neuropathic rats10,11. The plantar incision in rats (a model of postsurgical pain) is also characterized by the reduced withdrawal threshold to mechanical12 and thermal13 stimuli. In the present study, the analgesic action of continuous intrathecal morphine and loperamide has been compared in a rat model of postsurgical pain using mechanical and thermal stimuli. Further, loperamide analgesia and associated tolerance following continuous intrathecal infusion, have also been evaluated.

Materials and Methods

Animals—Adult male Sprague-Dawley rats (275-350 g) were obtained from the Central Animal Facility of the All India Institute of Medical Sciences, New Delhi. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee, All India Institute of Medical Sciences, New Delhi (No. Inv. 468/08-IAEC). Animals were kept under standard laboratory conditions in 12 h/12 h light-dark cycles with food and water provided ad libitum.

Intrathecal catheterization—Rat was anaesthetized in an induction chamber with 4% isoflurane in a mixture of air and oxygen (1:1) and placed in a stereotaxic frame with the head stabilized by ear bars. Isoflurane concentration was then reduced to 1.5–2% and maintained throughout the surgical procedure.

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The intrathecal catheter was surgically implanted as described by Yaksh and Rudy with modification. About 1 cm long midline incision was made in the skin on the back of neck. The interscutularis muscle attached to the occipital crest was detached about 3 mm lateral on either side of midline by blunt dissection. Interscutularis muscle was retracted caudally to expose 3-4 mm of the cisternal membrane (atlanto-occipital membrane). A 2 mm transverse incision was made on the membrane. When the membrane was cut, cerebrospinal fluid (CSF) appeared. Excess CSF was absorbed with cotton-tipped applicator. The caudal edge of incision in cisternal membrane was lifted with dura hook. A catheter made of polyurethane (8.5 cm long, 0.36 mm outer diameter and 0.18 mm inner diameter) (0007740, Durect Corporation, Cupertino, CA, USA) was pushed gently beneath the membrane and glided caudally for 8.5 cm into the intrathecal space. The caudal end of the catheter was placed close to the lumbar enlargement and the out-dwelling end was connected to coiled catheter (PE 50) with the help of 22G stainless steel connector. The other end of coiled catheter was connected to the flow moderator of osmotic minipump (2ML2, Durect Corporation CA, USA) (Fig. 1). Osmotic minipump was filled with drug and primed overnight at 37 °C before implantation. It delivered drug at a rate of 5 µL/h. Coiled catheter contained normal saline, which was delivered over a period of about 3 days (observed as recovery period). Saline administration was followed by the test drug on day 4th of osmotic pump implantation. Catheterized rats were subjected to stepping placing and righting reflex. Those animals exhibiting motor deficits like paralysis of hind paw were excluded from the study. Out of 54, only 48 (about 90%) rats were found suitable for further study. Post-mortem catheter placement in the vicinity of lumbar enlargement was examined by injection of 20 µL radio-opaque contrast (Lipidol). After catheter placement, topical neomycin ointment was applied over incision and perioperative ketoprofen (3 mg/kg, im) was given to relieve pain.

**Plantar incision**—On 4th day after implantation of osmotic pump, rats were subjected to Hargreaves paw withdrawal test at every 2 h intervals to note the appearance of analgesia. The animals showing drug-induced analgesia were then used for plantar incision. The rat model of postsurgical pain used for this study was the plantar incision model, standardized previously by Brennan et al. Surgical area was prepared with absolute alcohol and povidone iodine solution scrub. Under isoflurane anesthesia, a midline incision of 1 cm length was made using a number 11 surgical blade, through the skin and fascia. Incision started 0.5 cm proximal from the heel towards the toe. The underlying plantaris muscle was lifted up with a curved forceps and the muscle was incised longitudinally. Tip of the forcep was inserted through the cut in plantaris muscle and the blades separated for 0.5 cm. Finally, skin was apposed by 2 mattress sutures using 5-0 nylon with a FS-2 needle.

**Drugs and administration protocol**—Morphine sulphate (Verve Health Care Ltd. Pune, India) was diluted with normal saline. Loperamide hydrochloride (Sigma-Aldrich Chemicals Ltd, MO, USA) was dissolved in vehicle made of polyethylene glycol, saline and ethanol in a ratio of 2:2:1 as described previously. Animals received continuous intrathecal doses (0.5 µg/h, 1 µg/h and 2 µg/h) of morphine and loperamide till 7th day post-plantar incision. Separate group of animals were considered for each dose (n=6). Also, paw incision in rats with intrathecal saline (n=6) and vehicle (n=6) administration were studied. The paw withdrawal latency to thermal and mechanical stimuli was measured at 3 h, 1 day, 3 day, 5 day and 7 day after plantar incision.

**Thermal hyperalgesia**—Primary afferent and spinal sensory neurons have been reported to respond to brief pulse of intense infrared radiation. Thermally-evoked

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![Fig. 1—Setup for chronic intrathecal drug infusion. Pump filled with drug was connected to coiled catheter and the other end of coiled catheter was connected to polyurethane catheter through stainless steel connector. 1=Osmotic pump (2ML2, Durect Corporation CA, USA), 2=Flow moderator, 3=Coiled Catheter (Polyethylene 50), 4=Stainless steel (22 G) Connector, 5=Polyurethane (ID-0.38 mm and OD-0.99 mm), 6=Polyurethane (ID-0.44mm and OD-0.61mm) and 7=Polyurethane (ID -0.20 mm and OD - 0.35 mm). ID = Inner Diameter and OD = Outer Diameter.](image-url)
nociception in postsurgical rats to radiant heat was examined using Hargreaves paw-withdrawal apparatus (37370, Plantar Test Apparatus, UGO Basile, Italy). To begin the test, rats were placed individually in a clear perspex enclosures situated on an elevated glass floor and acclimatized for 30 min. Radiant heat source was focused directly under the plantar surface near the midplantar area of rat’s hind paw. When the rat feels pain and withdraws its paw, a sensor switched off both the heat source and the timer. Thus, the paw withdrawal latency (PWL) was automatically recorded. Thermal stimulus evoked behaviours such as paw withdrawal and even licking of the paw. The radiant heat source was adjusted to keep the baseline latencies between 8-11 s. A cut-off time of 20 s was preset to prevent possible tissue damage. Rats were subjected to three test trials separated by 5 min interval and the values were averaged to yield mean paw withdrawal latency. PWLs were converted to % Maximum Possible Effect [MPE (%) = (post-drug latency – baseline)/(cut-off time – baseline latency)x100]

Mechanical allodynia—Postoperative mechanical allodynia induction test was performed using calibrated nylon monofilaments, called von Frey Filaments (North Coast, CA, USA). Rats were acclimatized in clear perspex boxes kept over the wire mesh floor (12 x 12 mm grid). Filaments were applied perpendicular to the paw and medial to the incision for 5 s in up and down order, starting with 2 g until a withdrawal response occurred or cutoff was reached. The lower and upper cutoffs were 0.4 g and 15 g respectively. Withdrawal of the hind paw from the filament was scored as a response. When no response was obtained, the next stiffer filament in the series was applied in the same manner. If a response was observed, the next lighter fiber was applied. Testing proceeded until 4 fibers had been applied after the first one causing a withdrawal response. PWLs were transformed to % MPE by the formula: [MPE (%) = ((PWL post-drug – PWL pre-drug)/(15 – PWL post-drug) x 100)]

Statistical analysis and data presentation—Data are presented as mean ± SE. Behavioural test data were converted to % MPE. Graph plotting and statistical analysis was done using Graphpad Prism, Version 5.03 (Graph Pad Software, Inc. San Diego, CA, USA). Statistical evaluation was performed by non-parametric Kruskal-Wallis test, followed by Dunn’s Multiple comparison post-hoc tests. The significance was considered at P < 0.05.

Results
Paw incision induced significant heat hyperalgesia and mechanical allodynia in rats. Both morphine and loperamide reversed pain behaviour in response to radiant heat and mechanical stimuli produced by Hargreaves apparatus and von Frey Filaments respectively. There was no significant difference in PWLs between paw incision (saline) and vehicle treated group.

Estimation of post-surgical mechanical allodynia after chronic intrathecal drug administration—Lower dose (0.5 µg/h) of neither morphine nor loperamide reversed mechanical allodynia whereas 1 µg/h dose of morphine produced significant analgesia at 3 h and 3 day compared to paw incision group. Loperamide, at 1 µg/h dose reversed mechanical allodynia till 3rd day post-plantar incision. Morphine, (2 µg/h), produced significant analgesia till 3rd day post incision. However, loperamide, (2 µg/h), reversed mechanical allodynia throughout the testing regime (Fig. 2A and 2B).

Fig. 2—Mechanical stimulation of the incision site on rat’s right hind paw with von Frey filaments and determination of the antinociceptive effect of chronic intrathecal doses of (A) morphine and (B) loperamide. PWLs were transformed to % MPE. *P<0.05 vs. paw incision (Pw-In). b denotes 1 µg/h and c denotes 2 µg/h dose.
Evaluation of post-surgical thermal hyperalgesia after chronic intrathecal drug administration - Compared to paw incision group, morphine (0.5 µg/h) significantly decreased heat hyperalgesia at 3 h and day 1 post incision whereas same dose of loperamide produced analgesia at 3 h and day 3. Rats treated with 1 µg/h morphine and loperamide exhibited significantly less heat hyperalgesia at 3 h, day 1 and day 3 post-incision. Moreover, the same dose of loperamide reversed thermal hyperalgesia also on 5th day. Morphine (2 µg/h) reversed thermal hyperalgesia till 5th day whereas (2 µg/h) loperamide treated animals exhibited significantly less pain over the full time (till 7th day) course compared to paw incision (Fig. 3A and 3B).

Discussion

Spinal administration of analgesics is widely used in clinical settings to treat pain. Different types of Ca\(^{2+}\) channels and opioid receptors are present in the dorsal horn of the spinal cord. Direct administration of opioids into the intrathecal space enables interaction with these opioid receptors. Intrathecal opioids act through presynaptic effect on primary afferents by an inhibition of the opening of voltage gated calcium channels (VGCCs) and by postsynaptic hyperpolarization mediated by increased potassium conductance.

The analgesic action of chronic intrathecal opioids (morphine and loperamide) was compared after plantar incision in rats. The doses of morphine and loperamide chosen for the study were based upon pilot study and dose-response obtained from acute intrathecal administration. Morphine and loperamide produced dose dependent analgesia with the same onset time of antinociception to thermal and mechanical stimuli at 3 h post plantar incision. The same onset time of loperamide and morphine analgesia indicate equal penetration from CSF to the dorsal horn. Opioids with high lipid solubility diffuse more rapidly than opioids with polar characteristics. Therefore, loperamide being lipophilic could act more rapidly than morphine. On the other hand, morphine has high water solubility, thus its levels in CSF remains longer than in plasma.

In the present study, drugs with different polarity and pharmacokinetic profile had same onset of action, possibly due to influence of other factors. Morphine has low protein binding efficacy (about 30-40%) and short half-life (2-3 h) whereas loperamide has high protein binding capacity (about 97%) and longer half life (9-14 h). The lowest dose (0.5 µg/h) of neither morphine nor loperamide reversed mechanically evoked pain but the higher doses (1 µg/h and 2 µg/h) of both the drugs could reverse mechanically evoked pain. This is because the degree of neuronal blockade depends on the amount and concentration of local drug used and the properties of the axon. Morphine and loperamide, at 2 µg/h dose reversed mechanical allodynia till 3rd day and 7th day respectively, post-plantar incision. The highest dose (2 µg/h) of morphine reversed thermal nociception till 5th day, however, same dose of loperamide produced effect till 7th day. Lipid soluble drugs generally have a longer duration of action due to decreased clearance by local blood flow and increased protein binding. It has also been reported that, lipophilic local anesthetics, such as bupivacaine remains for a longer time in the tissue surrounding the nerve than a less lipophilic agent such as lidocaine. This partially explains the longer duration of action of loperamide which is more lipophilic (cLogP = 4.7).
than morphine (cLogP = 0.6). The highest dose of morphine produced analgesia only till 3rd and 5th day post-incision in von Frey and Hargreaves test, respectively, possibly due to the development of tolerance.

The antinociceptive effect of morphine decreased after continuous administration, whereas loperamide analgesia was maintained till 7th day post-planter incision. A lack of tolerance to loperamide could be observed and same has been reported after repeated peripheral administration. The difference in pharmacokinetic profile of morphine and loperamide may have influenced the development of tolerance. Furthermore, chronic morphine administration activates spinal NMDA receptors, which plays a crucial role in the development of morphine tolerance and blockade of the same significantly attenuates tolerance. Loperamide on the other hand, blocks Ca2+ ion entry through N-methyl-D-aspartate-receptor, which probably delays or attenuates development of its tolerance. Also, nefiracetam, an opener of calcium channels reversed loperamide analgesia but not that of morphine.

Moreover, thermal and mechanical hyperalgesia rely on activation of two different intracellular cascades in the spinal cord. In thermal hyperalgesia spinal activation of NMDA receptors, translocation of protein kinase C, and production of nitric oxide and cGMP occurs. In contrast, mechanical hyperalgesia cause co-activation of spinal AMPA and metabotropic glutamate receptors, activation of phospholipase A2 and production of cyclooxygenase products. These intracellular cascades may have induced difference in the analgesic action of same drug in postsurgical thermal hyperalgesia and mechanical allodynia tests.

In postsurgical pain, both morphine and loperamide showed analgesia against incision induced thermal hyperalgesia and mechanical allodynia. Morphine appears to induced tolerance after continuous intrathecal infusion. However, loperamide showed analgesia throughout the testing regime with no tolerance. This is the first report on absence of tolerance after chronic intrathecal infusion of loperamide. In postsurgical pain where chronic opioid is required, loperamide appears to be better than morphine. Loperamide, activates opioid receptors and may possibly blocks calcium channels, resulting into prolonged analgesia. Nevertheless, loperamide is also known to act through glutamate and GABA receptors, thus the action of intrathecal loperamide on these receptors in postsurgical pain cannot be ruled out.

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