Role of ion channel modifiers in reversal of morphine–induced gastrointestinal inertia by prokinetic agents in mice

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Prokinetic drugs like mosapride, domperidone etc, are used to treat gastrointestinal delay. Though the receptor-mediated actions of these agents have been studied, involvement of ion channels in reversing morphine-induced gastrointestinal inertia by prokinetic agents has not been explored. Charcoal meal test was used to measure small intestinal transit (SIT) in adult male Swiss albino mice. Animals were given ion channel modifiers and prokinetic drugs intragastrically. Reversal of morphine-induced gastrointestinal delay by mosapride was decreased significantly by CaCl2, minoxidil and glibenclamide. Similarly, domperidone’s effect on morphine was decreased by CaCl2, nifedipine, minoxidil and glibenclamide significantly. The results reveal that ion channel modifiers counteract the prokinetic effects of mosapride or domperidone.

Keywords: ATP gated K+ channels, Gastrointestinal delay, Ion channel modifiers, ‘L’ type voltage gated calcium channels, Morphine, Prokinetic drugs, Small intestinal transit

Opioids like morphine are one of the most potent analgesics from the ancient time and have been modified into a number of analogues and newer drug delivery systems have emerged for their administration. Though morphine does not cause end organ toxicities like NSAIDs and other conventional analgesics, it has been reserved mainly for terminal illness like cancer, chronic pain and post surgical pain for fear of addiction.

Morphine acts through μ and δ receptors and causes decrease in the cAMP level by inhibiting adenylyl cyclase. Apart from acting through receptors, morphine has been found to suppress voltage gated Ca2+ channels and enhance calcium dependent K+ channels resulting in decreased intracellular Ca2+ and increase in intracellular K+ respectively leading to gastrointestinal delay.

Prokinetic drugs like cisapride, mosapride, metoclopramide, erythromycin and domperidone have been used to overcome morphine-induced gastrointestinal delay both experimentally and clinically. These drugs act through different receptors and vary in their mechanisms of action and efficacy. However it is not known whether prokinetic drugs have any action through ion channels.

Interstitial cells of Cajal also known as intestinal pacemaker increases peristalsis by stimulating ‘L’ type Ca2+ channels and suppressing ATP gated K+ channels. Hence there is a probability that prokinetic drugs may also be acting by enhancing ‘L’ type Ca2+ channels and depressing ATP gated K+ channels. Thus there is a need to identify the possible ionic mechanisms involved in the reversal of morphine-induced gastrointestinal delay by prokinetic agents. This knowledge may help in the understanding of any untoward interactions between ion channel modifiers and prokinetics when taken together in presence of morphine as in cancer patients with co-morbid diseases such as hypertension, diabetes mellitus, gastroparesis, etc.

The present study has been undertaken to investigate the role of at least two openers and blockers of Ca2+ and K+ ion channels in the reversal of morphine-induced gastrointestinal inertia by prokinetic agents in mice. Two prokinetic agents namely mosapride and domperidone have been chosen since they are devoid of cardiac toxicity and extrapyramidal side effects as seen with other prokinetics like cisapride and metoclopramide.
Materials and Methods

Animals — The present study had three major groups namely drug control group, morphine treated group and drug interaction group. Each group had subgroups and 6 animals were included in each subgroup. Drug control group had eight subgroups in which the animals received only the individual drugs. Morphine treated group had six subgroups and each subgroup animals received morphine along with corresponding individual drugs. Under drug interaction group eight subgroups were present and received an ion channel modifier, a prokinetic and morphine. Randomly bred healthy adult male Swiss albino mice (132) weighing between 20-25 g were obtained from Central Animal House, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India. One week before the study, animals were procured from the central animal house in JIPMER and housed at departmental animal house in polypropylene cages under standard laboratory conditions. One day prior to the experiment, 6 animals belonging to each group were kept in separate cages with mesh bottom and fasted overnight with free access to water. Caged animals were housed at room temperature \((25\pm3^\circ C)\) with 12:12 hr light and dark cycle. Experiments were performed during the day time (0900 to 1230 hr). The experimental protocol was approved by JIPMER Institutional Animal Ethics Committee.

Drugs and chemicals — Calcium chloride (Fine Chem Industries, Chennai, India), domperidone (Torrent Pharmaceuticals, Ahmedabad, India), glibenclamide (Hoechst India Ltd, Mumbai, India), gum acacia (Hikasu Chemicals, Mumbai, India), minoxidil (Dr. Reddy’s Laboratory, Hyderabad, India), morphine sulphate (Government Opium and Alkaloid Works, Ghazipur, India), mosapride citrate (Alembic Chemical Works, Co. Ltd, Vadodara, India), nifedipine (Sigma Chemical company, Hyderabad, India), wood charcoal (Sarabai Chemicals, Baroda, India) were used. All drugs were dispersed in 5% w/v gum acacia mucilage in purified water and administered intragastrically (ig) to each animal using metal oral cannula connected to a glass syringe.

Measurement of small intestinal transit (SIT) — Charcoal meal marker was freshly prepared by dispersing 10% (w/v) wood charcoal in 5% (w/v) gum acacia mucilage in purified water and triturated well\(^9\). Each mouse received 0.3 ml marker orally through metal oral cannula. After 20 min animals were sacrificed by cervical dislocation, the abdomen was then cut opened; the leading front of marker was identified in the small intestine and tied immediately with a cotton thread to avoid movement of marker. The entire length of small intestine was isolated by cutting at pyloric and ileocaecal ends. The distance traveled by charcoal meal and the total length of the intestine were measured in cm(s). The SIT was expressed as percentage (%) of the distance traveled by the charcoal meal to length of the intestine.

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\text{Small intestinal transit (\%)} = \left( \frac{\text{distance traveled by charcoal meal (cm)}}{\text{total length of small intestine (cm)}} \right) \times 100
\]

Ion channel modifiers: CaCl\(_2\) (10 mg/kg; po), nifedipine (10 mg/kg; po), minoxidil (10 mg/kg; po) and glibenclamide (10 mg/kg; po).

Prokinetic agents: Domperidone (10 mg/kg; po) and mosapride (20 mg/kg; po).

Control groups — One group of mice was administered morphine subcutaneously (sc) through the nape of neck at a dose of 0.5 mg/kg and another group received equal volume of vehicle by the same route. The dose selection was based as per Ramasamy et al.\(^{10}\). Charcoal meal was administered 10 min after morphine or vehicle administration and SIT was determined after 20 min.

Prokinetic reversal groups — One group of mice received mosapride (20 mg/kg; po) and another group received domperidone (10 mg/kg; po) 15 min before morphine administration and SIT was determined after 45 min. The dose selection was based on the studies reported by Suchitra et al\(^6\).

Administration of calcium channel modifiers to study their effect on morphine-induced delay in SIT —
To study the effect of calcium channel modifiers on the action of prokinetic drugs, CaCl\(_2\) was used as a calcium channel agonist and nifedipine was used as a calcium channel blocker. One group of mice received calcium chloride (10 mg/kg; po) and another group received nifedipine (10 mg/kg; po). After 20 min
morphine was administered subcutaneously and the animals were sacrificed at the end of 50 min. The dose selection was based on previous studies. In the present study CaCl₂ was used as a calcium channel modifier instead of calcium channel agonist Bay K 8644 as the same could not be procured at that time. This is considered as one of the limitations of the present study.

Administration of potassium channel modifiers to study their effect on morphine-induced delay in SIT — To study the effect of potassium channel modifiers on the action of prokinetic drugs, minoxidil was used as a potassium channel opener and glibenclamide was used as a potassium channel blocker. One group of mice received minoxidil chloride (10 mg/kg; po) and another group received glibenclamide (10 mg/kg; po), 20 min before morphine administration and SIT was determined after 50 min. The dose selection was based on the studies of Venkatraman and Reddy.

Administration of calcium channel modifiers to study their effect on prokinetic-induced reversal of morphine-induced delay in SIT — Each group of mice received calcium chloride (10 mg/kg; po) or nifedipine (10 mg/kg; po), 5 min before mosapride or domperidone administration and later morphine was administered as mentioned earlier. The SIT was determined at the end of 50 min.

Administration of potassium channel modifiers to study their effect on prokinetic-induced reversal of morphine-induced delay in SIT — Each group of mice received minoxidil (10 mg/kg; po) or glibenclamide (10 mg/kg; po) 5 min before mosapride or domperidone administration and later morphine was administered as mentioned earlier. The SIT was determined after 50 min.

Statistical analysis — Statistical analysis was done by using Graph pad software, version 3.05, San Diego, USA. One way Analysis of Variance (ANOVA) was applied for small intestinal transit, followed by Dunnett’s multiple comparisons test. Values are expressed as mean ± SE. P<0.05 was considered as statistically significant.

Results

Effect of morphine on SIT — Morphine at the given dose caused significant decrease (55%) in SIT (P<0.001) when compared with vehicle treated group (Table 1).

Effect of prokinetic drugs on morphine-induced inhibition of SIT — Mosapride per se at the given dose increased SIT significantly (24.7%) compared with vehicle treated group (P<0.01) (Table 1). At this dose, mosapride reversed morphine-induced gastro-intestinal delay significantly (34.8%) (p<0.01). Domperidone per se at the given dose did not show any significant increase in SIT when compared with vehicle treated group. However, morphine-induced delay was reversed significantly by 47.3% (Table 1) when it was administered prior to morphine.

Effect of calcium chloride on morphine-induced inhibition of SIT — Calcium chloride per se did not show any significant change in SIT when given alone

<table>
<thead>
<tr>
<th>S No.</th>
<th>Drugs (mg/kg)</th>
<th>Alone</th>
<th>with morphine (0.5 mg/kg) sc</th>
<th>with morphapride (20 mg/kg) po + morphine (0.5 mg/kg) sc</th>
<th>with domperidone (10 mg/kg) po + morphine (0.5 mg/kg) sc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (0.3 ml) po</td>
<td>53.8±3.1</td>
<td>24.2±1.5</td>
<td>34.5±1.6</td>
<td>38.2±1.3</td>
</tr>
<tr>
<td>2</td>
<td>Morphine (0.5) sc</td>
<td>24.2±1.5**</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Mosapride (20) po</td>
<td>67.1±2.5*</td>
<td>34.5±1.6†</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Domperidone (10) po</td>
<td>58.9±1.8</td>
<td>38.2±1.3‡</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>CaCl₂ (10) po</td>
<td>52.8±2.7</td>
<td>29.8±2.0</td>
<td>21.9±1.6§</td>
<td>20.9±1.8#</td>
</tr>
<tr>
<td>6</td>
<td>Nifedipine (10) po</td>
<td>49.5±2.0</td>
<td>22.8±1.8</td>
<td>33.8±3.1</td>
<td>21.5±2.5#</td>
</tr>
<tr>
<td>7</td>
<td>Minoxidil (10) po</td>
<td>38.5±1.6**</td>
<td>23.4±1.4</td>
<td>22.4±2.1§</td>
<td>29.3±1.9#</td>
</tr>
<tr>
<td>8</td>
<td>Glibenclamide (10) po</td>
<td>44.8±1.1</td>
<td>26.7±1.9</td>
<td>22.4±1.1§</td>
<td>17.2±0.8#</td>
</tr>
</tbody>
</table>

P values: * <0.01 (compared to control); ** <0.001 (compared to control); † <0.01 (compared to morphine treated group); ‡ <0.001 (compared to morphine treated group); § <0.01 (compared to mosapride + morphine treated group); # <0.05 (compared to domperidone + morphine treated group); ## <0.001 (compared to domperidone + morphine treated group).
or with morphine (Table 1). However, CaCl₂ administered prior to mosapride and morphine, caused a significant delay in SIT by 36.5%. Similarly in domperidone and morphine treated group, CaCl₂ pretreatment caused a significant delay in SIT by 45.3% (Table 1).

Effect of nifedipine on morphine-induced inhibition of SIT — Nifedipine did not show any significant change in SIT when given alone, with morphine or with mosapride and morphine. However, in domperidone and morphine treated group, nifedipine pretreatment caused a significant delay in SIT by 43.7% (Table 1).

Effect of minoxidil on morphine-induced inhibition of SIT — Minoxidil per se caused significant decrease in SIT when compared with vehicle treated group \((P<0.001)\) (Table 1). When minoxidil was administered prior to morphine, it could not produce any significant change. However, when minoxidil was administered prior to mosapride and morphine it caused a significant delay in SIT by 35% compared with the group that was treated with mosapride and morphine. Similarly administered prior to domperidone and morphine treated group, minoxidil caused a significant delay in SIT by 23.3%.

Effect of glibenclamide on morphine-induced inhibition of SIT — Glibenclamide when given alone or prior to morphine no significant change in SIT was observed (Table 1). However, when glibenclamide was administered prior to mosapride and morphine, a significant delay in SIT by 55% was observed \((P<0.01)\). Similarly in domperidone and morphine treated group, glibenclamide caused a significant delay in SIT (55%) (Table 1).

Discussion

Motor activity in the gastrointestinal smooth muscle is related to membrane potential. Circular smooth muscle cells in the colon display rhythmic electrical oscillations called slow waves, which depend on the activity of both Ca²⁺ and K⁺ channels. Constipation is predominantly a disorder of motor function and may be due to neurogenic or iatrogenic causes. The most common iatrogenic cause of gastrointestinal delay is secondary to drugs like opioids.

Chronic administration of opioids like morphine causes constipation by decreasing gastric emptying and intestinal motility. It acts through μ and δ receptors and cause decrease in the cAMP level by inhibiting adenyl cyclase. Morphine increases Ca²⁺-dependent K⁺ conductance and causes hyperpolarisation of myenteric neurons and relaxation of intestinal smooth muscle. This in turn decreases Ca²⁺ influx during the action potential and interferes with the release of neurotransmitters. Moreover, morphine also has been found to suppress voltage gated Ca²⁺ channels. Thus by decreasing intracellular Ca²⁺, opioids decrease peristalsis resulting in gastrointestinal delay.

Mosapride citrate, acts through 5-HT₄ receptor and increase adenyl cyclase activity. It promotes GI motility by increasing release of acetylcholine from the neurons of the myenteric plexus.

Domperidone, a peripherally acting D₂ receptor antagonist increases gastric emptying by inhibiting the action of dopamine on the primary motor neurons in the myenteric plexus and overcomes the inhibitory action of dopamine on adenyl cyclase activity.

Animal studies have shown that mosapride and domperidone can be used to reverse morphine-induced gastrointestinal delay. Since at cellular level morphine inhibits Ca²⁺ channels and activates K⁺ channels, the purpose of this study is to see whether Ca²⁺ and K⁺ channels are involved in the reversal of opioid-induced gastrointestinal delay by prokinetic agents in mice.

In the present study, morphine caused a significant decrease in SIT, which is similar to previous studies. Mosapride produced a significant increase in SIT and also effectively reversed morphine-induced delay in SIT \((P<0.01)\) (Table 1). The result of this study was consistent with a similar study conducted in mice to find out the relative efficacy of different prokinetic agents.

Domperidone at a dose of 10 mg/kg did not produce any significant prokinetic effect (Table 1). However, when administered prior to morphine, it significantly reversed morphine-induced gastrointestinal delay \((P<0.001)\). The result obtained was similar to an earlier study conducted in mice.

CaCl₂ administration did not show any significant change in SIT either administered alone or prior to morphine (Table 1). However, when administered prior to mosapride and morphine, it significantly decreased SIT in mice \((P<0.01)\). Similar results were also obtained with domperidone. However, this result needs to be confirmed with calcium channel agonist Bay K 8644.
Nifedipine, a dihydropyridine, voltage gated ‘L’ type calcium channel blocker (10 mg/kg; po) did not produce any significant change in SIT when given alone or prior to morphine administration or prior to the administration of mosapride and morphine (Table 1). However, when nifedipine was administered prior to domperidone and morphine, it significantly decreased SIT ($P<0.001$). Therefore, in the presence of nifedipine, domperidone’s action seems to be reduced. We could not get any scientific explanation for this result. However, this could be probably due to the blockade of domperidone’s action through ‘L’ type calcium channels.

Minoxidil an ATP gated K$^+$ channel opener, produces relaxation of smooth muscle by hyperpolarisation. At a dose of 10 mg/kg, it significantly decreased SIT compared to the vehicle treated group ($P<0.001$) (Table 1). Minoxidil administration could not further lower the reduction in SIT produced by morphine. However, when minoxidil was given prior to mosapride or domperidone along with morphine, minoxidil further attenuated SIT significantly ($P<0.001$ and $P<0.05$). This shows that in presence of mosapride or domperidone, the effect of morphine has been enhanced by minoxidil, resulting in non reversal of SIT by mosapride or domperidone. This could be possibly explained by the synergistic action of minoxidil with morphine as both the drugs enhance intracellular K$^+$ concentration by acting through ATP gated K$^+$ channel and calcium dependent K$^+$ channel respectively. The resulting hyperpolarisation could be the reason for decrease in GI motility.

Glibenclamide, an ATP gated K$^+$ channel blocker in presence of mosapride and morphine, decreased SIT significantly ($P<0.01$). Similarly, when given prior to domperidone and morphine, glibenclamide decreased SIT significantly ($P<0.001$) (Table 1). Blockade of ATP gated potassium channel should have increased SIT but we got converse results. This may be probably due to blockade of only ATP gated K$^+$ channel and not calcium dependent K$^+$ channel through which morphine acts. Moreover these findings also suggest that concurrent treatment of glibenclamide with prokinetic drug and morphine counteracts the effect of prokinetics. However, the mechanism for this drug interaction is not known.

From the above findings, it can be stated that the prokinetic effects of mosapride and domperidone are probably mediated to a large extent through ‘L’ type Ca$^{2+}$ channels and not through ATP gated K$^+$ channels. This has been proved in the present study as domperidone-induced reversal of morphine’s action was altered in presence of nifedipine. However, the possible reason for decrease in SIT when prokinetic agents were administered with CaCl$_2$ could be due to an associated increase in intracellular Ca$^{2+}$ provided that prokinetic agents have some action through ‘L’ type Ca$^{2+}$ channels. The resulting high intracellular calcium could be causing enhanced Ca$^{2+}$-dependent K$^+$ conductance of morphine leading to hyperpolarisation and thus relaxation of smooth muscle of the intestine. This explanation is in light with the earlier study conducted in smooth muscle cells of the cerebral artery where increased intracellular calcium causes hyperpolarisation$^{20}$. Moreover, this result needs to be confirmed with calcium channel agonist Bay K 8644.

**Conclusion**

Prokinetic drugs like mosapride and domperidone are effective in overcoming morphine-induced gastrointestinal delay in mice. The ion channel modifiers viz., CaCl$_2$ (Ca$^{2+}$ channel modifier), minoxidil (ATP gated K$^+$ channel opener) and glibenclamide (ATP gated K$^+$ channel blocker) counteract the prokinetic effects of mosapride or domperidone. Similarly nifedipine (Ca$^{2+}$ channel blocker) counteracts the prokinetic effect of domperidone but did not alter the prokinetic effect of mosapride in presence of morphine. It can be hypothesized that nifedipine and mosapride can be concurrently used in cancer patients on morphine therapy with associated gastroparesis and hypertension. However, it needs to be proved by further clinical studies.

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