Research Results in Pharmacology

Research Results in Pharmacology 11(4): 199–204
UDC 615.212
DOI 10.18413/rrpharmacology.11.773

Research Article

Investigation of imidazole-4,5-dicarboxylic acid derivatives activity on visceral pain in mice

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Academic editor: Oleg Gudyrev ♦ Received 04 July 2025 ♦ Accepted 16 November 2025 ♦ Published 26 December 2025

Citation: Yakovleva EE, Kamalova MT, Yakovleva VG, Bychkov ER, Shabanov PD (2025) Investigation of imidazole-4,5-dicarboxylic acid derivatives activity on visceral pain in mice. Research Results in Pharmacology 11(4): 199–204. https://doi.org/10.18413/rrpharmacology.11.773

Abstract

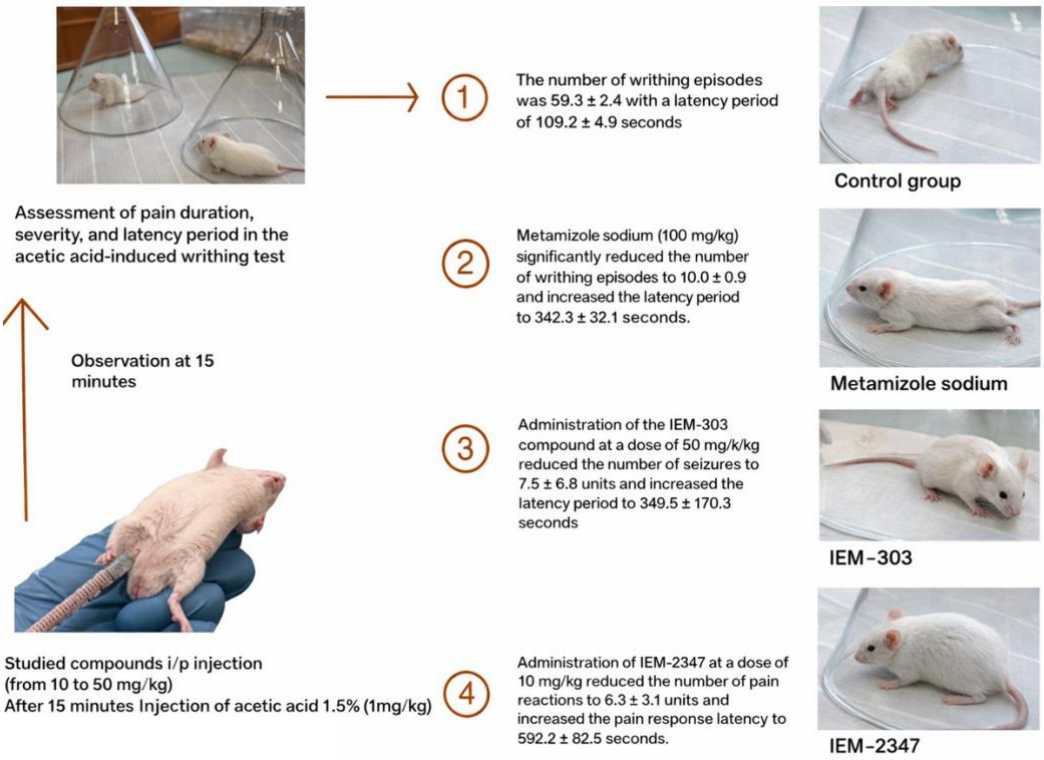
Introduction: Glutamate plays an important role in the modulation of nociception. Experimental studies on rodents have shown that mGluR1 receptor inhibitors demonstrate antinociceptive potential in acute pain models. New ligands of the glutamate NMDA-receptor complex are imidazole-4,5-dicarboxylic acid derivatives, which conformational rigidity allows increasing selectivity interaction and decreasing a number of side effects with the implementiation a high analgesic potential.

Materials and Methods: Intraperitoneal administration of algogen – 1.5% acetic acid solution –causes chemical pain irritation, manifested by specific animal movements called writhing. Fifteen minutes before the administration of 1.5% acetic acid solution, mice were intraperitoneally administered the experimental substances: IEM-303 at a dose of 50 mg/kg and IEM-2347 at doses of 10, 20, and 40 mg/kg. The control group of animals received a physiological solution in an equivalent volume, and the comparison group received metamizole sodium at a dose of 100 mg/kg. The analgesic effect was assessed by the ability of the drugs to reduce the number of writhings in comparison with the control group.

Results: The introduction of substance IEM-2347 at a concentration of 10 mg/kg led to a decrease in the number of writhings to 6.3 ± 3.1 units (by 89.4%), which significantly exceeds the indicators obtained in the group receiving metamizole sodium. With a further increase in IEM-2347 concentration to 20-40 mg/kg, complete suppression of the pain response was observed in 100% of animals.

Discussion: It can be assumed that the elongation of radicals in the benzene ring at nitrogen atoms has an effect enhancing the analgesic potential of the imidazole dicarboxylic acid derivative. **Conclusion**: High analgesic potential with toxicological safety of imidazole-4,5-dicarboxylic acid derivatives allows them to be considered as promising pain relievers.

Graphical Abstract



Keywords

glutamate, NMDA-receptor antagonists, imidazole-4,5-dicarboxylic acid derivatives, analgesic effect, visceral pain, mice

Introduction

Visceral pain occurs with pathological changes in internal organs and is the most common form of pain for which patients seek medical help (Kukushkin et al. 2011). Glutamate plays an important role in the nociception modulation. Numerous electrophysiological and behavioral studies have shown that mGlu receptors are directly involved in the pathogenesis of acute and chronic neuropathic pain (Mazzitelli et al. 2022). Experimental studies on rodents have shown that mGluR1 receptor inhibitors realize an antinociceptive potential in acute pain models (Mazzitelli et al. 2022; Hao et al. 2025). In the spinal cord, glutamate, being the main transmitter and affecting NMDA(N-methyl-D-aspartate)-receptors, promotes hyperalgesia and the formation of stable pain patterns (Davydova et al. 2007; Alyautdin et al. 2019; Yaksh et al. 1999). According to Urch et al. (2009), a pain impulse entering the posterior horns of the spinal cord causes depolarization, accompanied by the entry of sodium and calcium ions into the cells with the release of excitatory neurotransmitters – glutamate and substance P. The pathophysiological basis of central sensitization and increased sensitivity (wind-up phenomenon) in the dorsal horns of the spinal cord is the occurrence of slow postsynaptic potentials due to the release of glutamate, substance P, calcitonin gene-related peptide, which lead to excitation of NMDA-receptors, opening of potential-dependent calcium channels and prolonged depolarization of the postsynaptic membrane of neurons in the dorsal horns of the spinal cord (Bespalov et al. 2000; Barinov et al. 2010; Ovsyannikov et al. 2013; Farmer et al. 2009). It leads to the allodynia formation (the appearance of a pain even in response to non-painful stimuli) (Camilleri et al. 2000; Cervero et al. 2001). In this regard, the use of NMDA-antagonists in analgesic regimens, as well as the development of new compounds whose action is directed at the NMDA-receptor complex, is of particular interest. New ligands of the glutamate NMDA-receptor complex are derivatives of imidazole-4,5-dicarboxylic acid. The conformational rigidity of the molecules of imidazole-4,5-dicarboxylic acid derivatives allows increasing the selectivity interaction and decreasing a number of side effects with the

implementation a high analgesic potential. **The aim of the study** was to investigate the analgesic effect of new ligands of the glutamate NMDA-receptor complex – derivatives of imidazole-4,5-dicarboxylic acids – using the acetic acid-inducef writhing test in mice. Metamizole sodium, a reference analgesic widely used for the relief of acute pain in clinical practice, was used as the reference drug. A dose of 100 mg/kg of metamizole sodium was found to significantly reduce visceral pain in 100% of mice in preliminary experiments and is supported by literature data.

Materials and Methods

Animals

The experiments were performed on 36 male mice weighing 30-40 g, obtained from Rappolovo nursery of the Russian Academy of Medical Sciences (Leningrad Region, Russia). The animals were kept in standard plastic cages in vivarium conditions with free access to water and food at a temperature of 22±2 °C, and in the experiment they were divided into several groups (6 animals each). All the experiments were carried out in the autumn-winter period. The animals were kept in accordance with the rules of laboratory practice (GLP), regulatory documents "Sanitary Rules for the Device, Equipment and Maintenance of Vivarium" and the Order of the Ministry of Health and Social Development of the Russian Federation dated 23 August 2010 No. 708n "On Approval of the Rules of Laboratory Practice" (minutes of the Bioethics Committee of the Federal State Budgetary Scientific Institution "Institute of Experimental Medicine" No. 2/22 dated 06 April 2022).

The studied compounds

IEM-303 and IEM-2347 are the derivatives of imidazole-4,5-dicarboxylic acid synthesized in the S.V. Anichkov Department of Neuropharmacology, Institute of Experimental Medicine (Russia). The comparison drug is metamizole sodium (solution for intravenous and intramuscular administration, Biosintez, Russia).

Experimental model

The acetic acid-induced writhing test in mice is a model of visceral pain developing after intraperitoneal administration of algogen – 1.5% acetic acid solution causing chemical pain irritation manifested by characteristic animal movements (involuntary attacks of abdominal muscles constriction, back arching and hind limb extension) called writhings. The experimental substances were administered intraperitoneally fifteen minutes before administration of 1.5% acetic acid solution (1 mL/100 g): IEM-303 at a dose of 50 mg/kg (physiological solution was used as the solvent) to group 1 (6 mice) and IEM-2347 at doses of 10 mg/kg to group 2 (6 mice), 20 mg/kg to group 3 (6 mice) and 40 mg/kg to group 4 (6 mice) (physiological solution was used as the solvent). The control group of animals (group 5, 6 mice) received a physiological solution in an equivalent volume, the comparison group (group 6, 6 mice) received metamizole sodium (100 mg/kg, physiological solution was used as the solvent). Within 15 minutes after the introduction of algogen, the number of writhings was counted in each animal. The analgesic effect was assessed by the abilities to reduce the number of writhings in comparison with the control group. As an additional criteria allowing to evaluate pain sensitivity in animals, the latent period of the onset of writhings after the introduction of acetic acid solution was recorded (Mironov et al. 2013).

Statistical analysis

Statistical processing of the results was carried out using MS Excel 2010 and BioStat 2009. The normality of the data distribution was determined by the Shapiro-Wilk criterion. The reliability of the differences in values between the groups was defined using nonparametric criteria: Kruskal-Wallis and Fisher's exact criterion.

Results

The evaluation of the results of the study of the analgesic activity of new compounds – derivatives of imidazole-4,5-dicarboxylic acid revealed reliable differences between the investigational drugs (Table 1). The IEM-303 administration at a dose of 50 mg/kg led to a decrease in he number of writhings to 7.5 ± 6.8 units (by 87.4%) and an increase in the latent period duration to 349.5 ± 170.3 s (by 220.1%). The most impressive results were shown by the IEM-2347 introduction: when used at a dose of 10 mg/kg, the number of pain indicators decreased to 6.3 ± 3.1 units (by 89.4%), the latent period increased to 592.2 ± 82.5 s (by 442.3%),

which significantly exceeds the indicators obtained in the metamizole sodium group. When the dose of IEM-2347 was increased to 20-40 mg/kg, complete suppression of the pain reaction was observed in 100% of animals. Thus, a dose-dependent analgesic effect in the acetic acid-induced writhing test in mice was obtained for imidazole-4,5-dicarboxylic acid derivatives.

In the control group receiving saline, the average number of pain reactions was 59.3±2.4 units with a latent period of 109.2±4.9 s.

The administration of the standard analgesic metamizole sodium at a dose of 100 mg/kg demonstrated a decrease in the number of pain reactions to 10.0±0.9 units (a decrease of 83.1%) and an increase in the duration of latent period to 342.3±32.1 s (an increase of 213.4%).

Table 1. The efficiency of imidazole-4,5-dicarboxylic acid derivatives and metamizole sodium on visceral pain in the acetic acid-induced writhing test in mice (n=6; M±m; p≤0.01)

Substance	Dose, mg/kg	Number of writhings, units	Reduction in the number of writhings in comparison to that in the control group, %	Duration of latent period, s	Increase in the latent period duration in comparison to that in the control group, %
Control	-	59.3±2.4	-	109.2±4.9	-
Metamizole sodium	100	10.0±0.9	83.1	342.3±32.1*	213.4
IEM-303	50	7.5±6.8*	87.4	349.5±170.3*	220.1
IEM-2347	10	6.3±3.1*	89.4	592.2±82.5*	442.3
	20	0±0*	100	>900*	-
	40	0±0*	100	>900*	-

***Note*: *** p<0.01 in control group (Kruskal-Wallis criterion). For IEM-303, zero values were excluded (2 out of 6 mice did not respond). A latent period duration of >900 s indicates complete prevention and absence of writhings throughout the entire observation period.

Discussion

Numerous publications have demonstrated the analgesic effect of NMDA-receptor inhibitors. Thus, systemic administration of the mGluR5 antagonist MPEP (2-methyl-6-(phenylethynyl)-pyridine) prevented the development of pain in rats, recieved carrageenan, which leads to inflammatory hyperalgesia and edema development. Importantly, the administration of MPEP does not alter normal responses to painful mechanical or thermal stimulation in the paw compression and tail flick tests, indicating that the protective role of acute pain sensation in rodents was not impaired (Walker et al. 2001). However, this glutamate receptor type 5 antagonist does not reduce carrageenan-induced edema and has no effect on mechanical hyperalgesia or tactile allodynia in a rat model of neuropathic pain with partial ligation of the impaired sciatic nerve (Walker et al. 2001). These results confirmed similar findings by Dogrul et al. (2000), which showed that the glutamate blocker SIB-1757 reduced tactile allodynia and prevented 100% the development of thermal hyperalgesia in a spinal nerve ligation model at the L5/L6 level. It is also important to note that, unlike opioid analgesics or NSAIDs, NMDA inhibitors did not affect motor activity and coordination in the Rotarod test and did not exhibit ulcerogenic activity, which suggests a potential advantage of this class of pharmacological agents in terms of safety of their use (Mazzitelli et al. 2022). A reliable analgesic effect of NMDA blocker compounds was also demonstrated in models of acute pain caused by the introduction of formalin under the plantar aponeurosis, subcutaneous injection of interleukin-1β, and in a pain model with a skin incision (Ahn et al. 2005; Zhu et al. 2005). The results obtained in the course of the study confirm the literature data and demonstrate the presence of reliable analgesic activity of new ligands of the NMDA receptor complex – derivatives of imidazole-4,5-dicarboxylic acids – IEM-303 and IEM-2347 in acute pain models. Both imidazole-4,5-dicarboxylic acid derivatives have an amide structure and a similar chemical structure with an aromatic 5-membered ring. This results in similar pharmacokinetic properties for these prodrug-like compounds and equal permeability across the blood-tissue barrier. However, the radicals at 1 and 2 carbon atoms of these derivatives are represented by hydrocarbon chains of varying lengths: two methyl radicals in IEM-303 and a propyl and methyl radical in IEM-2347. Thus, the greatest analgesic activity was demonstrated for a new imidazole-4,5-dicarboxylic acid derivative with a longer length of both radicals at 1 and 2 carbon atoms of the aromatic ring, which apparently results in greater chemical affinity for the NMDA-receptor, modulation of ion current through the receptor channel, and inhibition of glutamate transmission, mediating the

antinociceptive potential of the studied pharmacological agent. A similar relationship between analgesic activity and the chemical structure of new NMDA ligands has been demonstrated in animal models of acute pain, such as the tail-flick test and the formalin test (Yakovleva et al. 2024). Taking into account a more pronounced analgesic effect of IEM-2347 at a dose of 20-40 mg/kg in mice (superior to the analgesic effect of metamizole sodium), compared to compound IEM-303 at a dose of 50 mg/kg, further detailed study of the analgesic potential of imidazole-4,5-dicarboxylic acid derivatives in various concentrations and analysis of the analgesic effect depending on the chemical structure and dose of the pharmacological agent is necessary.

Conclusion

1. New ligands of the NMDA receptor complex – derivatives of imidazole-4,5-dicarboxylic acids – IEM-303 and IEM-2347 exhibit a reliable analgesic effect.
2. The analgesic activity of IEM-2347 at a dose of 20-40 mg/kg when administered intraperitoneally to mice exceeds analgesic activity of the classical analgesic metamizole sodium (100 mg/kg), which is widely used in clinical practice.
3. The high analgesic potential along with the toxicological safety indicators of conformationally rigid molecules of selective inhibitors of the recognition site of the NMDA-receptor – derivatives of imidazole-4,5-dicarboxylic acid – allows us to consider them as promising effective and safe painkillers.

Additional Information

Conflict of interest

The authors declare the absence of a conflict of interests.

Ethics statement

The animals were kept in accordance with the rules of laboratory practice (GLP), regulatory documents ”Sanitary Rules for the Device, Equipment and Maintenance of Vivarium” and the Order of the Ministry of Health and Social Development of the Russian Federation dated 23 August 2010 No. 708n “On Approval of the Rules of Laboratory Practice” (minutes of the Bioethics Committee of the Federal State Budgetary Scientific Institution “Institute of Experimental Medicine” No. 2/22 dated 06 April 2022).

Data availability

All of the data that support the findings of this study are available in the main text.

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