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ABSTRACT

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CENTRAL ADJUVANTS AS MODIFIERS OF THE ANALGETIC ACTIVITY OF PARACETAMOL (EXPERIMENTAL SUBSTANCE)

Background. Multimodal analgesia is widely used to enhance pain control and reduce opioid consumption, yet experimental data on paracetamol (acetaminophen) combined with central adjuvant analgesics in visceral pain remain limited.

Purpose – to investigate and substantiate the effectiveness of multimodal combinations of paracetamol with central adjuvants to enhance analgesia in an experimental model of acute visceral pain.

Materials and Methods. The mice (n = 56) were randomly allocated into 8 experimental groups, 7 animals in each. In all experimental groups, a 0.75% acetic acid solution was administered intraperitoneum at a dose of 0.1 mL/10 g body weight as the algogenic agent. The groups differed in the selected multimodal analgesia regimen. Analgesic activity was assessed by the number of abdominal constrictions within 20 minutes after intraperitoneal acetic acid injection. Data were analysed using non-parametric statistics.

Results. Paracetamol monotherapy produced a moderate analgesic effect with a 35.2% reduction in writhing compared with the negative control but remained markedly inferior to morphine. Combinations with gabapentin, pregabalin, and amitriptyline did not significantly enhance analgesia versus paracetamol alone, showing only a modest trend toward further nociception reduction. In contrast, paracetamol with ketamine and paracetamol with dexmedetomidine combinations yielded a significant additional decrease in writhing (44.4% and 50.0% vs control, respectively; $p<0.05$ vs paracetamol), indicating a synergistic interaction between the central action of paracetamol and NMDA receptor blockade or α_2-adrenergic modulation. None

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of the combinations achieved the analgesic level of opioid therapy.

Conclusions. In a mouse model of acute visceral pain, paracetamol acts as a centrally active non-opioid analgesic with moderate efficacy. Meaningful potentiation of its analgesic activity is achieved only when combined with central adjuvants that exert complementary mechanisms ketamine and dexmedetomidine supporting their further translational evaluation in multimodal analgesic regimens.

Keywords: Acetaminophen, Analgesics, Adjuvant, Multimodal Analgesia, Pain, Visceral, Ketamine, Dexmedetomidine, Drug Synergism, Mice.

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ЦЕНТРАЛЬНІ АД'ЮВАНТИ ЯК МОДИФІКАТОРИ АНАЛЬГЕТИЧНОЇ АКТИВНОСТІ ПАРАЦЕТАМОЛУ (ЕКСПЕРИМЕНТАЛЬНЕ ОБҐРУНТУВАННЯ)

Актуальність. Мультимодальна аналгезія широко застосовується для підсилення контролю болю та зменшення опіоїдного навантаження, однак експериментальні дані щодо поєднання парацетамолу (ацетамінофену) з центральними ад'ювантними анальгетиками при вісцеральному болю залишаються обмеженими.

Мета роботи – дослідити й обґрунтувати ефективність мультимодальних комбінацій парацетамолу з центральними ад'ювантами для підсилення аналгезії в експериментальній моделі гострого вісцерального болю.

Матеріали та методи. Мишей (n = 56) рандомізовано розподілили на 8 експериментальних груп по 7 тварин у кожній. В усіх групах як альгогенний агент інтраперитонеально вводили 0,75% розчин оцтової кислоти в дозі 0,1 мл/10 г маси тіла. Групи відрізнялися між собою схемою мультимодальної аналгезії. Анальгетичну активність оцінювали за кількістю абдомінальних скорочень протягом 20 хвилин після інтраперитонеального введення оцтової кислоти. Дані аналізували з використанням непараметричних методів статистики.

Результати та їх обговорення. Монотерапія парацетамолом забезпечувала помірний аналгетичний ефект із зменшенням кількості «корчів» на 35,2% порівняно з негативним контролем, проте суттєво поступалася дії морфіну. Комбінації з габапентином, прегабаліном та амітриптиліну гідрохлоридом не призвели до статистично значущого посилення аналгезії порівняно з монотерапією парацетамолом, демонструючи лише помірну тенденцію до додаткового зниження ноцицепції. Натомість поєднання парацетамолу з кетаміном та парацетамолу з дексмедетомідином зумовлювали вірогідне додаткове зменшення кількості «корчів» (відповідно на 44,4% та 50,0% порівняно з контролем; $p<0,05$ відносно парацетамолу), що свідчить про синергічну взаємодію між центральною дією парацетамолу та NMDA-блокадою або α_2-

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адренергічною модуляцією. Жодна з комбінацій не досягла рівня аналгезії, притаманного опіоїдній терапії.

Висновки. У моделі гострого вісцерального болю в мишей парацетамол виступає як центрально активний неопіоїдний анальгетик із помірною ефективністю. Клінічно значуще підсилення його анальгетичної активності досягається лише при комбінуванні з центральними ад'ювантами, що реалізують комплементарні механізми дії, – кетаміном і дексмедетомідином, що обґрунтовує подальшу трансляційну оцінку таких поєднань у схемах мультимодальної аналгезії.

Ключові слова: парацетамол, мультимодальна аналгезія, ад'ювантні анальгетики, кетаміну гідрохлорид, дексмедетомідин, експеримент.

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INTRODUCTION

Multimodal analgesia (MMA) is regarded as a key strategy for pain control, particularly in surgery, oncology, traumatology, and intensive care, since combining drugs with different mechanisms of action allows simultaneous enhancement of the analgesic effect and reduction of opioid burden [1, 2]. The effectiveness of MMA is further supported by studies focused on the management of myofascial pain, vertebrogenic pain syndromes, and pathology of peripheral structures, which often accompany visceral pain and contribute to the development and maintenance of central sensitization [3]. Paracetamol (PAR) remains one of the most frequently prescribed non-opioid analgesics and is recommended by most clinical guidelines as a first- or second-line agent for acute and chronic pain, particularly in patients with concomitant somatic pathology and in older adults [4–6]. Despite its widespread use, paracetamol monotherapy is often insufficient for the control of moderate or severe pain [2, 7]. Considering that the pathogenesis of chronic pain includes autonomic dysregulation and neurocardiac dysfunction rather than being limited to a local problem in a joint, spine, or muscle, there is a need to individualize multimodal analgesic regimens [8].

Despite more than a century of clinical use, the molecular basis of paracetamol's analgesic action remains the subject of intensive scientific debate. Classical concepts were based on the hypothesis of cyclooxygenase (COX) inhibition in the central nervous system. However, it still has not been clarified which isoenzyme (COX-1 or a COX-2 variant) is the main pharmacological target of the drug [5].

Currently, the focus is increasingly shifting from peripheral COX inhibition toward central neuromodulatory mechanisms mediated by an active paracetamol metabolite formed in the CNS, which exerts antinociceptive effects via modulation of several key pain regulatory systems [5, 9]. In particular, it has been shown that activation of descending serotonergic 5-HT pathways is associated with suppression of nociceptive transmission at the level of the spinal cord [5, 10].

The body of evidence suggests that a leading role belongs to the formation of the biologically active metabolite AM404 [10, 11] in the central nervous system, which modulates the activity of vanilloid (TRPV1) and cannabinoid (CB1) receptors, thereby establishing a multimodal central mechanism of action [12, 13].

Clinical data on PAR as part of multimodal regimens are heterogeneous but generally support its rational use as an adjuvant. The addition of paracetamol to nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, or antipsychotic agents has been shown in most studies to reduce pain intensity and opioid requirements without a substantial increase in the incidence of adverse reactions [2, 6, 14].

At the same time, other investigators have demonstrated no additional benefit from including PAR in analgesic regimens, for example in patients receiving regional blocks during video-assisted thoracoscopic surgery or in those treated with combined metamizole-based analgesia in vitreoretinal surgery [15, 16]. Clinical examples of interventional techniques, such as adenohypophysis cryoablation for refractory pain syndromes, highlight the substantial role of the central component in shaping the pain phenotype [17]. Combat-related trauma, limb injuries, and acute soft tissue damage likewise demonstrate that effective analgesia requires a combination of agents with different targets, whereas monotherapy is almost always inferior to multimodal regimens [18].

Recent reviews on muscle relaxants with analgesic properties also emphasize the importance of their use

specifically within multimodal protocols, given their ability to affect central mechanisms of nociception [19]. These findings underline that the effect of PAR as a component of MMA largely depends on the type of nociceptive stimulus, the concomitant analgesic strategies, and likely the specific features of central sensitization in different pain models, particularly visceral pain.

One of the main limitations of paracetamol use is the risk of hepatotoxicity, which increases in a dose-dependent manner [20, 21]. A review by *Chidiac et al.* demonstrated that paracetamol overdose remains one of the leading causes of drug-induced acute liver failure worldwide, with a clearly dose-dependent risk of hepatotoxicity [20]. Under these circumstances, optimization of multimodal regimens using lower doses of paracetamol while maintaining or enhancing the analgesic effect becomes of practical importance.

Thus, particular interest lies in combinations of paracetamol with agents capable of specifically modulating central sensitization and descending inhibitory pathways. Ketamine, as an NMDA receptor antagonist, and dexmedetomidine, as a selective α_2-adrenoceptor agonist, have already gained a prominent role in anesthesiology and intensive care as tools within opioid-sparing strategies. Gabapentinoids and tricyclic antidepressants, through modulation of calcium channels, glutamatergic transmission, and descending monoaminergic systems, are likewise considered key central adjuvants in pain therapy [9, 10, 22].

However, despite the growing clinical experience with combinations of paracetamol and adjuvant analgesics, systematic preclinical data remain limited. The lack of a robust experimental foundation complicates the rational design of multimodal regimens and the reliable extrapolation of potential synergy into clinical practice. This underscores the need for targeted experimental evaluation of paracetamol combinations with central adjuvants, particularly under conditions of visceral nociception, in comparison with paracetamol monotherapy and opioid analgesia, which defines the rationale for the subsequent investigations.

The aim of this study was to investigate and substantiate the effectiveness of multimodal combinations of paracetamol with central adjuvants to enhance analgesia in an experimental model of acute visceral pain.

MATERIALS AND METHODS

The experimental study was conducted using 56 sexually mature male mice weighing 28–32 g. The animals were housed in a vivarium under standard conditions, receiving standard chow with free access to food and water (ad libitum), with stable microclimate parameters (temperature 22 ± 2 °C, 12/12 h light/dark cycle).

The classical model of visceral pain like the acetic acid writhing test was used to assess peripheral analgesic activity. Intraperitoneal (i.p.) injection of acetic acid triggers local release of inflammatory and pain mediators (bradykinin, histamine, serotonin, prostaglandins, leukotrienes). This causes a typical behavioural response: lateral stretching of the trunk, extension of the hind limbs, arching of the back, rubbing and licking of the abdominal wall, and rhythmic contractions of the anterior abdominal wall muscles with short relaxation intervals. The intensity of the nociceptive response was assessed by counting the number of writhes recorded within 20 minutes after injection of the algogenic stimulus [23, 24].

The hypothesis regarding potentiation of paracetamol's analgesic activity (AnA) was tested using a series of centrally acting adjuvants with distinct mechanisms of action: gabapentin (GAB), pregabalin (PREG), amitriptyline hydrochloride (AMI), ketamine hydrochloride (KTM), and dexmedetomidine (DMM).

The mice (n = 56) were randomly allocated into 8 experimental groups, 7 animals in each. The minimum group size (n=7) was determined based on the conventional design of primary screening studies using the acetic acid writhing model, expected between-group differences from published data, and the ethical principle of reduction in animal experimentation. Animals were randomly assigned to groups. Writhing counts were assessed by an observer blinded to treatment allocation. In all experimental groups, a 0.75% acetic acid solution was administered i.p. at a dose of 0.1 mL/10 g body weight as the algogenic agent. The groups differed in the selected MMA regimen (Table 1).

Dose planning, routes of administration, and drug combinations were designed taking into account current data on their pharmacokinetics, pharmacodynamics, and clinical efficacy [25]. Since all medicinal products used in the study (NSAIDs, non-opioid analgesics, and adjuvant analgesics) are registered in Ukraine and have been used in clinical practice for a long time, their average therapeutic doses for humans, as specified in official prescribing information and reference sources, were taken as a starting point. This approach enabled a scientifically justified translation of doses to the conditions of an animal experiment in laboratory mice [26].

Ethical Considerations. All necessary procedures were carried out in accordance with the national legislation of Ukraine ("On Protection of Animals from Cruel Treatment", No. 3447-IV), Directive 2010/63/EU of the European Parliament and of the Council, the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), current orders of the Ministry of Health and the Ministry of Education and

Science of Ukraine, as well as the ARRIVE 2.0 (2020) guidelines [27]. The comprehensive research programme was reviewed and approved by the Ethics and Bioethics Committee of the School of Medicine of V. N. Karazin Kharkiv National University (extract from Protocol No. 9 dated 6 May 2025).

Table 1 – Distribution of animals by groups and analgesic regimens (acetic acid writhing test), n = 56

Group	Group description	Analgesic therapy (dose, route, time before algogen administration)	n
I	Negative control	–	7
II	Opioid analgesia	Morphine (MORPH) 6.2 mg/kg, i.m., 10 min before	7
III	PAR monotherapy	PAR 131.6 mg/kg, i.m., 30 min before	7
IV	PAR + GAB	PAR 131.6 mg/kg, i.m., 30 min before; GAB 393.6 mg/kg, per os, 120 min before	7
V	PAR + PREG	PAR 131.6 mg/kg, i.m., 30 min before; PREG 65.8 mg/kg, per os, 60 min before	7
VI	PAR + AMI	PAR 131.6 mg/kg, i.m., 30 min before; AMI 19.7 mg/kg, i.m., 20 min before	7
VII	PAR + KTM	PAR 131.6 mg/kg, i.m., 30 min before; KTM 8.0 mg/kg, i.m., 20 min before	7
VIII	PAR + DMM	PAR 131.6 mg/kg, i.m., 30 min before; DMM 0.01 mg/kg, i.m., 60 min before	7

Methods of statistical analysis. Statistical analysis was performed using Microsoft Excel for primary data processing and graphical presentation of the results. Distribution normality was assessed using the Shapiro–Wilk test, which is appropriate for small sample sizes. Given the small number of animals per group and the non-normal distribution of the data, results were analyzed using nonparametric methods. Data are presented as median and interquartile range (Me [LQ; UQ]). Overall intergroup differences were assessed using the Kruskal–Wallis test, followed by post hoc pairwise comparisons using the Mann–Whitney U-test with adjustment for multiple comparisons. Differences were considered statistically significant at $p<0.05$. Results were graphically presented using box-and-whisker plots.

RESULTS

It was found that in the acetic acid writhing test, paracetamol (PAR) was characterized by an analgesic activity profile typical of non-opioid analgesics, with a predominant central mechanism of action. In animals that received PAR without an adjuvant, the number of abdominal constrictions was 35 [32; 38] at p=0.01, corresponding to a 35.2% reduction in pain reactions relative to the negative control group. At the same time, PAR monotherapy showed 62.9% lower efficacy compared with opioid analgesia (Fig. 1). These results confirm a stable yet moderate effectiveness of PAR in suppressing the pain response under conditions of acute visceral pain, where nociception with a central component predominates [28].

The combination of PAR with GAB (Fig. 1) was associated with minor changes in the parameter, which amounted to 36 [33; 37] with $p<0.001$ versus the negative control, corresponding to a 33.3% reduction in pain reactions, while maintaining a low level of efficacy compared with the opioid therapy group ($p<0.001$, 176.9%). The difference relative to PAR without an adjuvant (p=0.42; 2.9%) was not statistically significant. This indicates the absence of a substantial synergy between these agents, although the direction of change suggests a trend towards slight enhancement of the central analgesic effect. It is likely that the shared involvement of the GABAergic system limits the potential for additional potentiation in this combination [5, 9].

The combination of PAR with PREG (Fig. 1) led to a reduction in the number of abdominal constrictions to 33 [29; 37] with $p<0.001$ versus the control group, corresponding to a 38.9% decrease in pain responses. Opioid therapy demonstrated significantly higher AnA ($p<0.001$, 153.8%). Differences compared with PAR without an adjuvant (p=0.22; 5.7%) were not statistically significant, although the combination maintained a clear trend toward increased AnA. This may be explained by the cumulative action on central nociceptive inhibitory pathways, yet without a pronounced synergistic effect due to the similar direction of pharmacodynamic activity [29].

With the use of the PAR + AMI combination (Fig. 1), the parameter remained at 36 [30; 39] with $p<0.001$ versus the control, corresponding to a 33.3% reduction in pain responses, and again did not reach the level of opioid analgesia ($p<0.001$, 176.9%). The difference relative to PAR without an adjuvant (p=0.42; 2.9%)

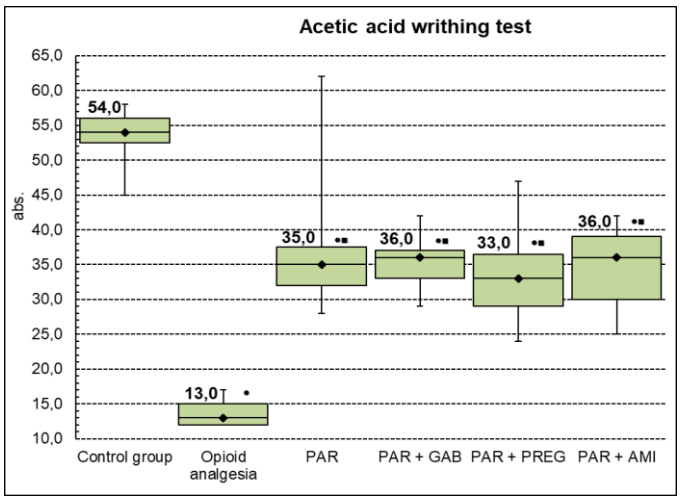

Figure 1 – Effect of gabapentin (GAB), pregabalin (PREG), and amitriptyline hydrochloride (AMI) on the analgesic activity of paracetamol (PAR) in the experimental model

Notes:

1. The data distribution is non-normal.

2. Boxes represent the interquartile range (25th to 75th percentiles); vertical whiskers indicate minimum and maximum values.

3. The horizontal line within the box represents the median.

4. ♦ inside the box denotes the meanvalue.

5. ● – $p < 0,05$ vs. the negative control group

6. ■ – $p < 0,05$ vs. the opioid therapy group

did not attain statistical significance. This indicates that combining two centrally acting agents with predominantly non-additive mechanisms does not provide a substantial increment in effect, but maintains stable AnA [13, 29].

A more pronounced reduction in the number of abdominal constrictions was observed with the combination of PAR and KTM (Fig. 2). The parameter decreased to 30 [25; 31] with $p<0.001$ versus the negative control group, corresponding to a 44.4% reduction in pain manifestations. The difference from PAR without an adjuvant was statistically significant (p=0.02; 14.3%), indicating a genuine enhancement of the effect due to the combination of the central action of PAR with NMDA receptor blockade by KTM [10, 13].

The most pronounced enhancement of PAR AnA was observed in the DMM group (Fig. 3), where the number of abdominal constrictions was 27 [23; 32] with $p<0.001$ versus the negative control, corresponding to a 50.0% reduction in pain reactions. The difference from PAR without an adjuvant was statistically significant (p=0.01; 22.9%), which confirms a genuine enhancement of the analgesic effect when combining the central action of PAR with the α_2-adrenomimetic activity of DMM [5, 9, 22].

None of the investigated analgesic combinations reached the level of the reference opioid therapy (Figs. 1–3).

Thus, the descending sequence 35→36→33→36→30→27 reflects the gradual enhancement of PAR AnA when combined with adjuvants of different mechanisms of action. The most pronounced and statistically confirmed effect was observed with the combinations of PAR + KTM (p=0.02; 14.3%) and PAR + DMM (p=0.01; 22.9%), indicating substantial synergy between central inhibition of nociceptive transmission and α_2-adrenoceptor modulation. The obtained results are fully consistent with the concept of MMA, whereby combinations of drugs with different pharmacological profiles provide deeper analgesia without increasing toxicity [20, 29].

PAR without adjuvants showed a value of 35 [32; 38], p=0.01, $p<0.001$, which reflects a moderate level of analgesia. The addition of GAB modified the response to 36 [33; 37] without a significant difference ($p>0.05$), while in combination with PREG it decreased to 33 [29; 37], and with AMI it was 36 [30; 39]. A marked reduction was observed with the combinations including KTM (30 [25; 31]; p=0.02) and DMM (27 [23; 32]; p=0.01), indicating that only combinations with adjuvants exerting a central mechanism of action produce a substantial enhancement of PAR AnA. This is consistent with known pharmacodynamic patterns whereby PAR exerts only a weak effect on peripheral nociception but potentiates central action via the serotonergic system.

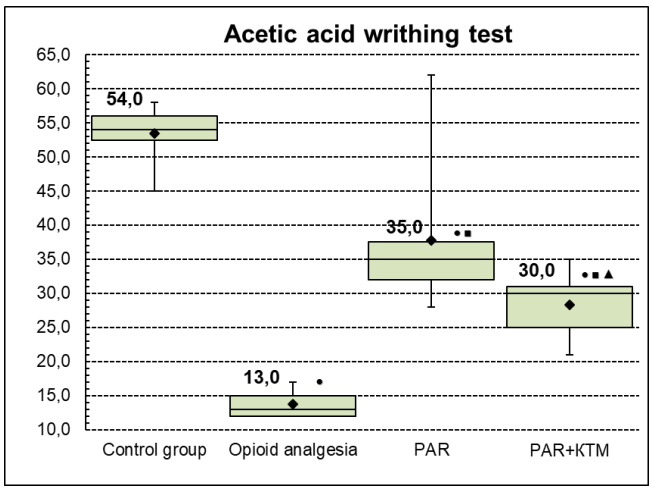

Figure 2 – Effect of ketamine hydrochloride (KTM) on the analgesic activity of paracetamol (PAR) in the experimental model

Notes:

1. The data distribution is non-normal.

2. Boxes represent the interquartile range (25th to 75th percentiles); vertical whiskers indicate minimum and maximum values.

3. The horizontal line within the box represents the median.

4. ♦ inside the box denotes the meanvalue

5. ● – p < 0,05 vs. the negative control group

6. ■ – p < 0,05 vs. the opioid therapy group

7. ▲ – p < 0,05 vs. he PAR monotherapy group

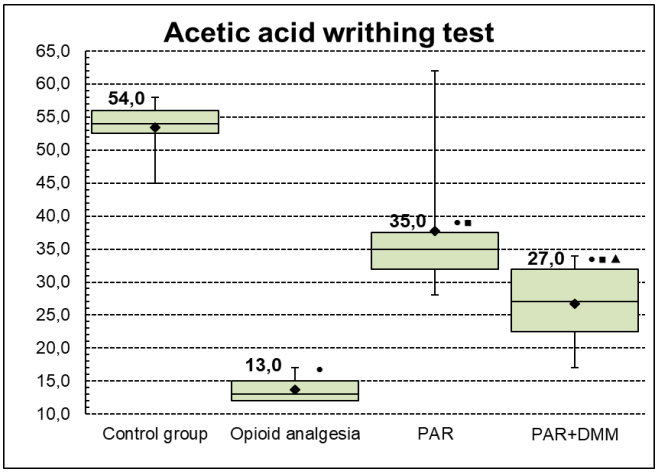

Figure 3 – Effect of dexmedetomidine (DMM) on the analgesic activity of paracetamol (PAR) in the experimental model

Notes:

1. The data distribution is non-normal.

2. Boxes represent the interquartile range (25th to 75th percentiles); vertical whiskers indicate minimum and maximum values.

3. The horizontal line within the box represents the median.

4. ♦ inside the box denotes the meanvalue

5. ● – p < 0,05 vs. the negative control group

6. ■ – p < 0,05 vs. the opioid therapy group

7. ▲ – p < 0,05 vs. he PAR monotherapy group

DISCUSSION

Our study demonstrates that in the selected experimental model of visceral pain, PAR provides stable but moderate AnA, which is markedly inferior to the reference opioid analgesia with morphine. The addition of centrally acting adjuvants from different pharmacological classes revealed fundamentally distinct interaction profiles: from the absence of a significant enhancement of the effect (GAB, PREG, AMI) to statistically significant synergy when combined with KTM and DMM.

The obtained results are in good agreement with current concepts of the mechanism of action of PAR as a centrally acting analgesic. In our study, combinations of PAR with GAB, PREG, and AMI did not demonstrate a statistically significant reduction in the number of abdominal constrictions compared with monotherapy, although they retained a trend toward a modest additional decrease in nociception. Gabapentinoids and tricyclic antidepressants are considered first-line agents for the treatment of neuropathic pain, where they exert their effects through modulation of calcium channels, glutamatergic transmission, and noradrenergic and serotonergic pathways [29, 30, 31]. However, in models of acute visceral pain based on acetic acid, their analgesic potential is less pronounced and often requires high doses or combination with other agents to achieve a clinically meaningful effect [25].

The lack of a markedly pronounced synergy between paracetamol and these adjuvants in our study can be interpreted as a manifestation of a plateau effect, where combining agents with partially overlapping mechanisms of action does not produce a substantial increase in analgesia due to saturation of shared neurotransmitter and receptor pathways. This is consistent with clinical data indicating that combination regimens based on gabapentinoids and antidepressants show advantages mainly in chronic, neuropathic, and nociplastic pain, but are less convincing in acute nociceptive pain of visceral origin [25, 32, 33].

In contrast to gabapentinoids and AMI, the combination of PAR with KTM resulted in a significant additional reduction in the number of abdominal constrictions (44.4% relative to the control) and a statistically significant difference compared with PAR monotherapy, indicating a genuine enhancement of analgesic activity and the presence of a synergistic component. KTM, as a non-competitive NMDA receptor antagonist, plays a key role in preventing and attenuating central sensitization, particularly under conditions of repeated nociceptive stimulation and prolonged visceral pain. Contemporary reviews emphasize its effectiveness as a component of multimodal analgesia, especially at low (subanesthetic) doses in the postoperative period, where it reduces pain intensity and opioid requirements [9, 34].

In addition, clinical data have shown that a combination of intravenous paracetamol and ketamine decreases the severity of postoperative pain and the need for opioid therapy compared with tramadol-based combinations [35]. In the context of our model, where acetic acid induces both peripheral inflammatory and pronounced central components of visceral nociception, NMDA blockade by ketamine logically potentiates the central mechanisms of paracetamol action, providing deeper suppression of nociceptive transmission [10].

Thus, combinations of agents with complementary rather than merely additive mechanisms of action appear to be the most promising.

The most effective MMA regimen in our study was the combination of PAR with DMM, as evidenced by a 50% reduction in the number of abdominal constrictions relative to the control and a significant enhancement of AnA compared with PAR monotherapy. DMM, a highly selective α_2-adrenoceptor agonist, exerts potent analgesic, sedative, and sympatholytic effects and is increasingly incorporated into Enhanced Recovery After Surgery (ERAS) protocols as a component of opioid-sparing strategies [11]. Animal studies confirm the ability of DMM to attenuate nociception, including in models of visceral pain, as well as to improve hemodynamic stability during anesthesia [29, 31].

At the same time, clinical studies demonstrate substantial heterogeneity of effects. For example, the addition of PAR to certain MMA regimens (e.g., in the setting of regional blocks) does not improve outcomes [15]. This partly explains the intermediate nature of the effect in the present model: PAR alone reduces the intensity of visceral nociception, but does not achieve the level of opioid analgesia.

Interpretation of the observed differences should also consider that the tested agents were administered via different routes (intramuscular vs oral) and at different time intervals before algogen administration. These regimens were selected to approximate the expected onset of pharmacological action for each compound; however, differences in absorption and time to peak effect could have influenced the behavioural endpoint.

The acetic acid writhing model used in this study is a classical tool for assessing analgesic activity in acute visceral pain. It is sensitive to a wide range of both opioid and non-opioid analgesics [25, 30, 31]. However, several limitations must be taken into account, including the predominance of afferent stimulation from the peritoneum, the absence of process chronification, and the substantial influence of the inflammatory component. Although the acetic acid-induced writhing test is a validated and widely used model of acute visceral

nociception, it remains a behavioural paradigm and therefore may be influenced by observer-dependent factors. Future studies should incorporate instrumental methods such as electromyography or automated video-based behavioural analysis to further improve objectivity. Accordingly, extrapolation of these results to clinical settings such as postoperative, oncologic, or functional visceral pain should be approached with caution.

Nevertheless, the findings of this study support the general concept of MMA, whereby rational combinations of agents with divergent central mechanisms can provide a clinically meaningful enhancement of analgesia without straightforward dose escalation or global opioid up-titration [2].

CONCLUSIONS

The obtained data experimentally confirm the role of paracetamol as a centrally acting non-opioid analgesic in a model of acute visceral pain, where it provides moderate analgesic activity that is markedly inferior to the effect of opioid analgesia with morphine.

Combinations of paracetamol with gabapentin, pregabalin, and amitriptyline hydrochloride did not demonstrate a statistically significant enhancement of analgesia in the experiment, showing only a trend toward a modest additional reduction of nociception.

The combination of paracetamol with ketamine provided a significant enhancement of the analgesic response, reflecting synergy between the AM404-mediated central action of paracetamol and NMDA receptor blockade aimed at reducing central sensitization in visceral pain.

The most pronounced augmentation of analgesia was observed with the combination of paracetamol and dexmedetomidine, confirming substantial synergy between the central mechanism of action of paracetamol and α_2-adrenergic modulation of descending inhibitory pathways.

PROSPECTS FOR FUTURE RESEARCH

The experimental data obtained support the rationale for further investigation of multimodal regimens based on paracetamol in combination with ketamine and dexmedetomidine, including dose–response analyses, extension to additional pain models (chronic and nociplastic pain), and clinical trials in patients with a pronounced visceral pain component, taking into account the safety limitations of paracetamol.

AUTHOR CONTRIBUTIONS

Matvieienko M.S. - conceptualization and study design, execution of experimental research, statistical analysis of the obtained data, drafting of the main manuscript text, and formulation of conclusions.

Hladkykh F.V. - statistical analysis of the obtained data, drafting of the main manuscript text, and formulation of conclusions.

Chyzh M.O. - participation in experimental research, contribution to the discussion of the results, and manuscript editing.

Gogiya M.O. - participation in experimental research, statistical analysis of the obtained data.

Markov O.V. - contribution to the discussion of the results, and manuscript editing.

CONNECTION WITH OTHERS CIENTIFIC WORKS

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CONFLICT OF INTEREST

The authors of the manuscript hereby consciously declare the absence of any actual or potential conflicts of interest with pharmaceutical companies, biomedical device manufacturers, or other organizations whose products, services, or financial support may be related to the subject matter of the submitted materials or who may have sponsored the conducted research.

ETHICAL CONSIDERATIONS

All necessary procedures were carried out in accordance with the national legislation of Ukraine (“On Protection of Animals from Cruel Treatment”, No. 3447-IV), Directive 2010/63/EU of the European Parliament and of the Council,

the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), current orders of the Ministry of Health and the Ministry of Education and Science of Ukraine, as well as the ARRIVE 2.0 (2020) guidelines [27]. The comprehensive research programme was reviewed and approved by the Ethics and Bioethics Committee of the School of Medicine of V. N. Karazin Kharkiv National University (extract from Protocol No. 9 dated 6 May 2025).

ARTIFICIAL INTELLIGENCE DISCLOSURE

The authors declare that no artificial intelligence (AI)-based technologies were used in the writing of the manuscript. AI tools were used exclusively for language editing and manuscript refinement.

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