



Multimodal Combinations of Diclofenac With Central Adjuvants in Adjuvant Arthritis: Effects on Mechanical Hyperalgesia and Central Sensitisation

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Abstract

Background/Aim: Chronic inflammatory pain is a complex multifactorial condition involving sustained immune, neuronal and glial activation, with persistent alterations in both peripheral and central nociceptive processing. Aim of the study was to investigate the antinociceptive efficacy of diclofenac sodium alone and in combination with centrally acting adjuvants in a rat model of adjuvant arthritis.

Methods: Adjuvant arthritis was induced in male rats using complete Freund's adjuvant. Animals were allocated to six groups: intact control, untreated arthritis, diclofenac monotherapy and diclofenac combined with gabapentin, pregabalin or amitriptyline. Treatment was administered from day 14 to day 28. Mechanical nociceptive thresholds were assessed using the Randall–Selitto test.

Results: Adjuvant arthritis caused a marked reduction in mechanical nociceptive threshold by day 14. By day 28, diclofenac monotherapy partially restored pain thresholds, whereas combinations with gabapentin and pregabalin produced significantly greater recovery compared with diclofenac alone. The amitriptyline combination showed a moderate effect that did not reach conventional statistical significance. These findings indicate a greater contribution of central mechanisms in the later phase of inflammatory pain.

Conclusions: Diclofenac-based multimodal therapy, particularly in combination with gabapentinoids, provides superior antinociceptive efficacy compared with NSAID monotherapy in chronic inflammatory pain. These findings support the relevance of targeting both peripheral and central mechanisms in persistent arthritis-related hyperalgesia.

Key words: Diclofenac; Arthritis, experimental; Central nervous system sensitisation; Hyperalgesia; Gabapentin; Pregabalin; Analgesia.

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Introduction

Chronic inflammatory pain is a complex multifactorial condition involving sustained immune, neuronal and glial activation, with persistent alterations in both peripheral and central nociceptive processing.^{1, 2} Contemporary concepts

consider chronic pain not only as a symptom of tissue injury, but also as an independent pathological state driven by immunoinflammatory signalling, autoimmune mechanisms, mitochondrial dysfunction and central sensitisation.^{3, 4} In rheu-

matoid arthritis and related immune-mediated inflammatory arthropathies, pain may persist even when inflammatory activity is adequately controlled, reflecting the contribution of neuroplastic and centrally mediated mechanisms to its maintenance.⁵⁻⁹

Nonsteroidal anti-inflammatory drugs (NSAIDs) remain a key component of inflammatory pain treatment because they inhibit cyclooxygenase activity and reduce prostaglandin-mediated peripheral sensitisation. Diclofenac sodium (D-Na) is one of the widely used NSAIDs with established analgesic and anti-inflammatory effects.^{10,11} However, NSAID monotherapy is often insufficient in chronic pain syndromes, particularly when central sensitisation or multimorbidity is present. In addition, long-term NSAID use is limited by dose- and duration-dependent gastrointestinal, cardiovascular and renal risks.¹²⁻¹⁶ These limitations support the need for NSAID-sparing and mechanism-based multimodal analgesic strategies.

Current understanding of arthritis-related pain extends beyond peripheral inflammation. Central sensitisation, characterised by increased excitability of spinal and supraspinal nociceptive pathways, may develop as a result of prolonged nociceptive input, N-methyl-D-aspartate (NMDA) receptor activation, imbalance between excitatory and inhibitory neurotransmission and neuroimmune interactions.¹⁷⁻¹⁹ Experimental and clinical studies indicate that central sensitisation can sustain allodynia and hyperalgesia independently of ongoing inflammatory activity and may reduce responsiveness to standard anti-inflammatory therapy.^{20,21}

In this context, multimodal analgesia, based on the combination of agents with different mechanisms of action, represents a pathophysiologically justified approach to chronic inflammatory pain.^{22,23} Centrally acting adjuvant analgesics, including gabapentinoids such as gabapentin and pregabalin, as well as tricyclic antidepressants such as amitriptyline hydrochloride, modulate neuronal excitability, calcium channel activity and monoaminergic inhibitory pathways.²⁴⁻²⁷ These mechanisms may be particularly relevant when chronic inflammatory pain acquires neuropathic-like or centrally sensitised features. Evidence from systematic reviews and meta-analyses suggests potential analgesic effects of antidepressants and gabapentinoids in chronic musculoskeletal pain, although the results remain heterogeneous and

depend on the pain model, treatment duration and combination regimen used.²⁶⁻²⁹

Experimental models of chronic inflammatory pain, particularly the adjuvant arthritis (AA) model in rats, are widely used for studying pain mechanisms and evaluating combined analgesic strategies. This model reproduces key features of immune-mediated arthritis, including persistent inflammation, mechanical hyperalgesia, immune activation and systemic manifestations, making it suitable for assessing pharmacological approaches targeting both peripheral and central components of pain.^{5,6,30,31} In the AA model, the pain phenotype evolves over time: early stages are predominantly associated with peripheral inflammation and prostaglandin-mediated sensitisation, whereas later stages increasingly involve spinal hyperexcitability, neuroimmune interactions and neuroplastic changes.

Despite the theoretical rationale for combining NSAIDs with centrally acting adjuvants, comparative experimental data on diclofenac-based multimodal regimens in established adjuvant arthritis remain limited. Therefore, investigation of D-Na in combination with pregabalin (PREG), gabapentin (GAB) and amitriptyline hydrochloride (AMI) may help clarify whether centrally acting adjuvants enhance antinociceptive efficacy during the chronic phase of inflammatory pain and whether such effects are time-dependent.

Aim of this study was to investigate the analgesic efficacy of D-Na monotherapy and its multimodal combinations with centrally acting agents (pregabalin, gabapentin and amitriptyline hydrochloride) in a rat model of chronic inflammatory pain induced by adjuvant arthritis, with a focus on time-dependent changes in mechanical hyperalgesia as an indirect behavioural indicator of peripheral and central pain mechanisms.

Methods

Adjuvant arthritis model

To model chronic inflammatory pain, an experimental AA model was employed. AA in rats was induced by subplantar injection of complete Freund's adjuvant (CFA; *Thermo Fisher Scientific*, USA) into the left hind paw between the second

and third digits. This method induces a localised inflammatory response and is a widely accepted approach for reproducing autoimmune arthritis in rodents. The model is characterised by the development of hyperalgesia, oedema, hyperaemia and involvement of contralateral limbs, thereby reproducing key pathophysiological features of human rheumatoid arthritis.^{30, 32}

The course of experimentally induced arthritis was characterised by sequential phases of the pathological process. The reactive phase (up to days 10–15) included an early subphase (0–8 h), during which local hyperaemia, oedema, erythema, increased tissue temperature and hyperalgesia were observed as early as 1.5–2 h after CFA injection.³³

During the subacute subphase (days 1–6), signs of systemic inflammatory response developed, including increased body temperature, leucocytosis with neutrophilic shift, elevated erythrocyte sedimentation rate (ESR) and general intoxication (lethargy, reduced activity, decreased appetite). The transitional subphase (days 7–14) was characterised by generalisation of the process with involvement of contralateral limbs and proximal joints, further progression of hyperalgesia and increased joint swelling. The acute phase (days 15–30) manifested as progressive joint oedema, pronounced hyperaemia, local hyperthermia and reduced motor activity.^{33, 34}

The day of CFA administration was considered day 0 of the experiment. Treatment was initiated on day 14 and continued until day 28, corresponding to the period of a stabilised immune-inflammatory response. The investigated drugs were administered daily either intramuscularly or intragastrically depending on their pharmacological class. This design allowed assessment of therapeutic effects under conditions of an established chronic inflammatory process.³³⁻³⁵

Experimental design and animal groups

The study evaluated the analgesic activity of D-Na and its combinations with gabapentin (GAB), pregabalin (PREG) and amitriptyline (AMI). The AA model was used to assess NSAID efficacy under conditions of chronic autoimmune inflammation, taking into account both peripheral (prostaglandin-mediated) and central sensitisation mechanisms. Combined administration of D-Na with centrally acting agents enabled evaluation of potential synergistic effects within a multimodal analgesia framework.

Doses, routes of administration and drug combinations were determined based on pharmacokinetic and pharmacodynamic data as well as clinical efficacy. The intervals between administration of diclofenac and centrally acting adjuvants were selected according to the expected onset of pharmacodynamic activity and available pharmacokinetic data for each agent. However, the lack of direct pharmacokinetic confirmation in the present study should be acknowledged as a limitation. Initial reference doses corresponded to average therapeutic human doses with subsequent interspecies dose conversion using standard scaling factors.^{36, 37}

Six experimental groups were formed (Table 1):

- Group I – intact animals;
- Group II – AA-induced rats without treatment (control);
- Group III – AA + D-Na;
- Group IV – AA + D-Na + GAB;
- Group V – AA + D-Na + PREG;
- Group VI – AA + D-Na + AMI.

Drugs were administered once daily from day 14 to day 28 after AA induction according to the study protocol. Analgesic activity was assessed on days 0, 14 and 28 based on changes in pain patterns and the duration of pain response latency.

Table 1: Experimental groups

Group	n	Experimental conditions
I	7	Intact rats receiving intramuscular (im) 0.9 % NaCl (1.0 mL/kg) daily from days 14–28
II	7	AA-induced rats (control), receiving im 0.9 % NaCl (1.0 mL/kg) 60 min prior to CFA administration
III	7	AA + D-Na (8.0 mg/kg, im, days 14–28)
IV	7	AA + D-Na (8.0 mg/kg, im) + GAB (72.1 mg/kg, intragastrically), administered separately with a 60 min interval
V	7	AA + D-Na (8.0 mg/kg, im) + PREG (24.0 mg/kg, intragastrically), administered separately with a 30 min interval
VI	7	AA + D-Na (8.0 mg/kg, im) + AMI (3.2 mg/kg, im), administered separately with a 20 min interval

AA: adjuvant arthritis; D-Na: diclofenac; PREG: pregabalin; GAB: gabapentin; AMI: amitriptyline hydrochloride; CFA: complete Freund's adjuvant;

Assessment of pain sensitivity

Mechanical nociceptive threshold was assessed using the Randall–Selitto test with a paw pressure analgesia meter (*Ugo Basile*, Italy; maximum load 750 g).³⁸ Pressure was applied to the dorsal surface of the hind paw via a blunt probe at a constant rate of 39.2 g/s. The nociceptive threshold (g) was defined as the force at which withdrawal reflex and/or vocalisation occurred. To prevent tissue injury, the maximal applied force was limited to 375 g.^{39,40} This method allows quantitative assessment of mechanical hyperalgesia as an integral indicator of nociceptive response, incorporating both spinal and supraspinal components.

Animals and housing conditions

The study was conducted on adult male outbred white rats ($n = 42$) weighing 180–220 g. Animals were randomised into six groups ($n = 7$ per group) based on baseline pain sensitivity assessed using the tail immersion test (Table 1). No formal a priori sample size calculation was performed. The group size was selected based on previous experimental studies using the adjuvant arthritis model and Randall–Selitto testing. This limitation is acknowledged and future studies should include formal power calculation based on expected effect size.

Randomisation was performed using a computer-generated sequence. Allocation concealment was ensured by using numbered, opaque, sealed envelopes opened only after inclusion of the animal and completion of baseline testing. Outcome assessment was performed by an investigator blinded to group allocation. The person conducting the Randall–Selitto test was not involved in treatment administration and had no access to the randomisation sequence. Animals were housed under standard vivarium conditions: temperature 22 ± 2 °C, 12/12 h light/dark cycle, with free access to food and water (*ad libitum*).

Statistical analysis

Statistical analysis was performed using Microsoft Excel 2016. Normality of distribution was assessed using the Shapiro–Wilk test ($n < 50$) and homogeneity of variances using Levene's test. For normally distributed data, Student's t-test was applied; for paired comparisons, the Wilcoxon signed-rank test was used. Data are presented as mean $\pm$ standard error of mean ($M \pm m$), with 95 % confidence intervals (CI) where appropriate.

Results

The AA model was associated with the development of chronic inflammatory pain, as evidenced by a characteristic decrease in the nociceptive threshold in the Randall–Selitto test, reflecting the formation of persistent mechanical hyperalgesia. The overall dynamics of nociceptive threshold changes across the study period are summarised in Table 2 and illustrated in Figures 1 and 2.

At baseline (day 0), all experimental groups demonstrated comparable nociceptive threshold values, indicating adequate baseline homogeneity before induction of the pathological process. The mean threshold in intact rats was 311.4 ± 5.1 g. Similar values were observed in the AA control group and in all treatment groups, ranging from 305.7 ± 4.3 g to 314.3 ± 4.3 g. No statistically significant differences between groups were detected at this stage ($p > 0.05$).

By day 14, all AA-induced groups showed a pronounced reduction in mechanical nociceptive threshold compared with baseline values and intact animals. In untreated AA rats, the threshold decreased to 151.4 ± 4.0 g, corresponding to an approximately 50.5 % reduction from baseline ($p = 0.009$). A comparable decrease was observed in the D-Na monotherapy group, where the threshold reached 147.1 ± 6.1 g, representing a 52.8 % reduction from baseline ($p = 0.009$). At this time point, D-Na monotherapy did not differ significantly from untreated AA controls ($p > 0.05$).

Similar day 14 patterns were observed in the combination therapy groups. The nociceptive threshold was 154.3 ± 6.1 g in the D-Na + GAB group, 150.0 ± 4.9 g in the D-Na + PREG group and 157.1 ± 5.2 g in the D-Na + AMI group. Although these values were slightly higher than those observed in untreated AA controls in some groups, none of the treatment regimens produced a statistically significant improvement compared with the AA control group on day 14 ($p > 0.05$). Thus, comparative analysis at this stage confirmed a significant difference between intact animals and all AA-induced groups ($p < 0.001$), while no significant differences were detected between untreated AA animals and treated AA groups.

By day 28, the differences between treatment

Table 2: Mechanical nociceptive threshold in the Randall–Selitto test in rats with adjuvant arthritis (Mean $\pm$ m (95 % CI), g; n = 7 per group)

Time point	Group I (Intact)	Group II (AA control)	Group III (AA + D-Na)	Group IV (AA + D-Na + GAB)	Group V (AA + D-Na + PREG)	Group VI (AA + D-Na + AMI)
Day 0	311.4 $\pm$ 5.1 (301.5–321.4)	305.7 $\pm$ 4.3 (297.3–314.1)	311.4 $\pm$ 4.0 (303.5–319.3)	314.3 $\pm$ 4.3 (305.9–322.7)	308.6 $\pm$ 3.4 (301.9–315.2)	312.9 $\pm$ 4.2 (304.6–321.1)
Day 14	314.3 $\pm$ 5.7 (303.1–325.5)	151.4 $\pm$ 4.0 (143.5–159.3)*	147.1 $\pm$ 6.1 (135.3–159.0)*	154.3 $\pm$ 6.1 (142.3–166.3)*	150.0 $\pm$ 4.9 (140.4–159.6)*	157.1 $\pm$ 5.2 (146.9–167.4)*
Day 28	305.7 $\pm$ 5.7 (294.5–316.9)	204.3 $\pm$ 7.5 (189.6–219.0)*	224.3 $\pm$ 10.4 (203.8–244.7)*†	268.6 $\pm$ 8.0 (252.9–284.2)*†‡	261.4 $\pm$ 7.4 (247.0–275.9)*†‡	248.6 $\pm$ 5.1 (238.6–258.5)*†

AA – adjuvant arthritis; D-Na – diclofenac sodium; GAB – gabapentin; PREG – pregabalin; AMI – amitriptyline hydrochloride; CI – confidence interval.

* $p < 0.05$ vs intact group at the corresponding time point; † $p < 0.05$ vs AA control at day 28; ‡ $p < 0.05$ vs AA + D-Na at day 28; Mean $\pm$ m: mean $\pm$ standard error of mean;

groups became more evident. In intact animals, nociceptive threshold values remained stable throughout the observation period, confirming the absence of relevant time-dependent changes unrelated to AA induction. In untreated AA controls, the nociceptive threshold remained substantially reduced and reached 204.3 ± 7.5 g, corresponding to a 33.2 % decrease from baseline. Although this value was higher than that observed on day 14, it remained markedly lower than in intact animals, indicating persistence of mechanical hyperalgesia.

D-Na monotherapy resulted in a moderate restoration of nociceptive threshold by day 28. The mean threshold in this group increased to 224.3 ± 10.4 g, which was significantly higher than in untreated AA controls ($p < 0.001$). Compared with day 14, this represented a 52.4 % increase, indicating a time-dependent antinociceptive effect of D-Na during prolonged treatment. However, the threshold in the D-Na group remained lower than in intact animals, suggesting incomplete recovery of mechanical pain sensitivity.

The greatest restoration of nociceptive threshold was observed in the groups receiving D-Na in combination with gabapentinoids. In the D-Na + GAB group, the threshold increased to 268.6 ± 8.0 g by day 28. This value was significantly higher than in untreated AA controls ($p < 0.001$) and also significantly exceeded the effect of D-Na monotherapy ($p = 0.006$). The increase from day 14 to day 28 reached 74.1 %, representing the most pronounced improvement among the treatment groups.

A similar effect was observed in the D-Na + PREG group. By day 28, the nociceptive threshold reached 261.4 ± 7.4 g, which was significantly higher than in untreated AA controls ($p < 0.001$) and significantly exceeded the D-Na monotherapy group ($p = 0.013$). The increase relative to

day 14 was 74.3 %, closely corresponding to the effect observed in the D-Na + GAB group. These results indicate that both gabapentin- and pregabalin-based combinations produced a stronger recovery of mechanical nociceptive threshold than D-Na alone.

The D-Na + AMI combination produced a less pronounced, but still evident, increase in nociceptive threshold. By day 28, the mean threshold in this group reached 248.6 ± 5.1 g, which was significantly higher than in untreated AA controls ($p < 0.001$). Compared with D-Na monotherapy, the threshold was higher by 16.6 %; however, this difference did not reach conventional statistical significance ($p = 0.058$). The increase from day 14 to day 28 was 58.2 %, indicating a moderate restoration of mechanical pain threshold.

Longitudinal analysis showed that all treatment groups demonstrated significant increases in nociceptive threshold between days 14 and 28 ($p = 0.009$). The magnitude of recovery differed between regimens: the largest increases were observed in the D-Na + GAB and D-Na + PREG groups, followed by the D-Na + AMI group and D-Na monotherapy. Between-group comparisons at day 28 confirmed a statistically significant advantage of the gabapentin- and pregabalin-based combinations over D-Na monotherapy, whereas the amitriptyline-based combination showed a borderline effect (Figure 1).

Overall, the results demonstrate that AA induction caused persistent mechanical hyperalgesia, while prolonged treatment with D-Na partially restored nociceptive thresholds. The addition of gabapentin or pregabalin provided a significantly greater antinociceptive effect than D-Na alone, particularly by day 28. The amitriptyline combination showed a moderate improvement, but its advantage over monotherapy remained at the threshold of statistical significance (Figure 2).

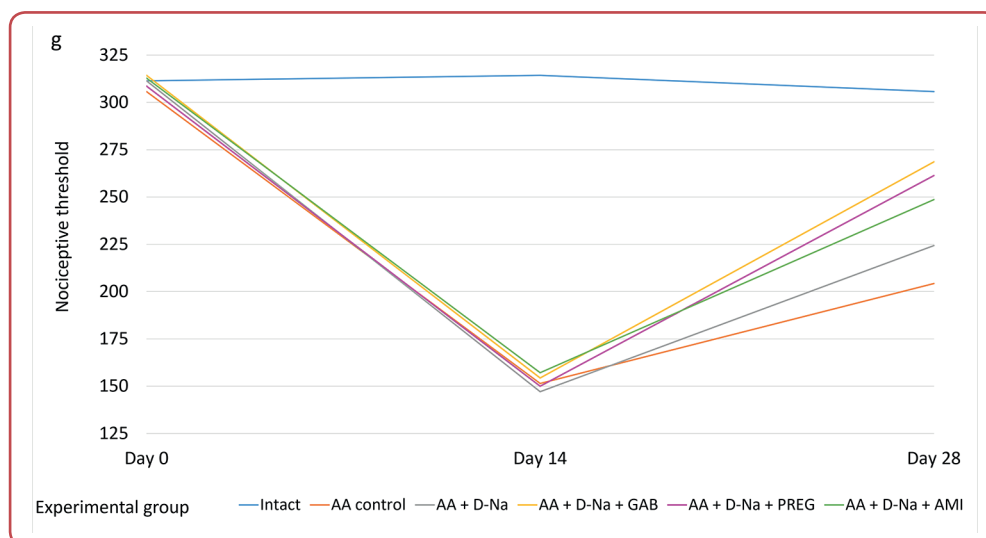


Figure 1: Time-dependent changes in mechanical nociceptive threshold in rats with adjuvant arthritis

AA: adjuvant arthritis; D-Na: diclofenac; PREG: pregabalin; GAB: gabapentin; AMI: amitriptyline hydrochloride;

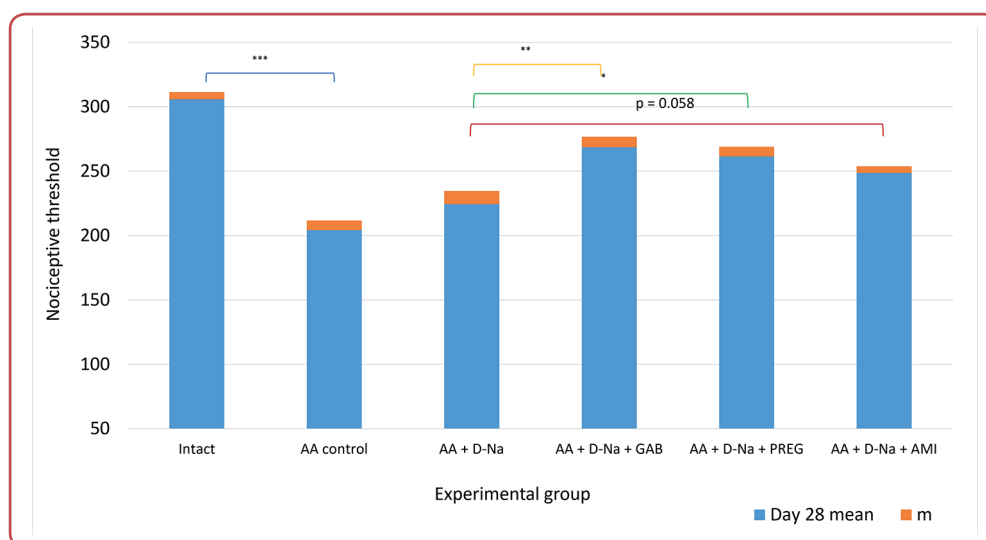


Figure 2: Mechanical nociceptive threshold on day 28 after diclofenac-based mono- and combination therapy

AA: adjuvant arthritis; D-Na: diclofenac; PREG: pregabalin; GAB: gabapentin; AMI: amitriptyline hydrochloride; m: standard error of mean;

Discussion

The main finding of this study is that diclofenac-based combination therapy, particularly with gabapentin or pregabalin, produced greater restoration of mechanical nociceptive threshold than diclofenac monotherapy in the late phase of adjuvant arthritis. This effect was most evident on day 28, whereas on day 14 none of the treatment regimens showed a statistically significant advantage over untreated AA controls. Thus, the results indicate a time-dependent difference in treatment response and support the rationale for

combining an NSAID with centrally acting adjuvant analgesics in persistent inflammatory pain. The AA model reproduced a stable phenotype of chronic inflammatory pain characterised by pronounced mechanical hyperalgesia. By day 14, all AA-induced groups demonstrated a marked reduction in nociceptive threshold compared with baseline and intact animals. At this stage, D-Na monotherapy did not significantly improve mechanical threshold compared with untreated AA controls and combination regimens showed only

non-significant trends toward improvement. This pattern is consistent with the concept that arthritis-related pain is not determined solely by local inflammation but also involves neuro-immune interactions, cytokine-mediated signaling and plasticity of nociceptive pathways.¹⁻³ In chronic inflammatory states, these mechanisms may limit the efficacy of isolated cyclooxygenase inhibition, particularly when sensitisation of the nociceptive system has already developed.^{1,2}

By day 28, treatment-related differences became more pronounced. D-Na monotherapy produced a moderate but significant increase in nociceptive threshold compared with untreated AA controls, indicating partial restoration of mechanical pain sensitivity. However, the greatest recovery was observed in the D-Na + gabapentin and D-Na + PREG groups. These combinations significantly exceeded D-Na monotherapy, suggesting that gabapentinoids enhanced the antinociceptive effect of diclofenac during the later phase of AA. The delayed emergence of this superiority may reflect a growing contribution of centrally mediated mechanisms to persistent hyperalgesia, although this interpretation should be considered indirect because molecular or electrophysiological markers of central sensitisation were not assessed in the present study.

The potentiation of D-Na effects by gabapentinoids is mechanistically plausible. GAB and PREG act mainly through binding to the $\alpha 2\delta$ subunit of voltage-gated calcium channels, thereby reducing the release of excitatory neurotransmitters in nociceptive pathways.^{36,38} This mechanism is relevant to conditions in which prolonged nociceptive input promotes spinal hyperexcitability and neuroplastic changes.¹⁷⁻¹⁹ Clinical and experimental data also support the role of central sensitisation in rheumatoid arthritis and other chronic musculoskeletal pain conditions, where pain may persist despite incomplete correlation with inflammatory activity.^{20,21} Therefore, the stronger effect of D-Na + GAB and D-Na + PREG in the present study is consistent with the broader concept of multimodal analgesia, in which peripheral anti-inflammatory therapy is combined with agents targeting central amplification of pain.^{28,29}

The effect of the D-Na + AMI combination was less pronounced. Although this regimen improved nociceptive threshold compared with untreated AA controls, its advantage over D-Na monotherapy remained at the threshold of statistical signifi-

cance. This finding requires cautious interpretation. Tricyclic antidepressants exert analgesic effects mainly through enhancement of descending serotonergic and noradrenergic inhibitory pathways, as well as modulation of spinal and supraspinal pain processing.^{25,26} However, their effects may depend on dose, duration of exposure and the specific pain phenotype. Evidence from systematic reviews of antidepressants in chronic joint pain also indicates heterogeneous efficacy across populations and study designs.²⁶ In the present study, the borderline result for D-Na + AMI may therefore reflect limited statistical power, an insufficient exposure period, or a less prominent role of monoaminergic mechanisms in this experimental setting compared with calcium channel modulation by gabapentinoids.

The temporal profile of the analgesic response is an important aspect of the present findings. On day 14, all AA groups remained highly hyperalgesic and between-group differences were not significant. By contrast, on day 28, D-Na monotherapy improved mechanical threshold, while its combinations with GAB and PREG provided significantly greater recovery. This pattern supports the assumption that the mechanisms maintaining pain in AA evolve over time: early phases are more closely related to active peripheral inflammation and prostaglandin-mediated sensitisation, whereas later phases may increasingly involve spinal hyperexcitability, neuroimmune interactions and neuroplastic changes.¹⁷⁻²⁰ The behavioural results of this study are compatible with this model, although direct confirmation of central sensitisation would require additional molecular, histological, or electrophysiological assessment.

The translational relevance of these findings lies in the potential value of NSAID-sparing multimodal analgesic strategies. NSAIDs, including diclofenac, are widely used for inflammatory pain but are associated with gastrointestinal, cardiovascular and renal risks, particularly with prolonged use, higher doses, older age and multimorbidity.¹²⁻¹⁶ In this context, combinations that improve analgesic efficacy may theoretically allow reduction of NSAID exposure while maintaining pain control. However, this implication should be interpreted carefully because the present study did not directly assess dose reduction, adverse effects, or long-term safety. Moreover, gabapentinoids also require cautious use in clinical populations, especially in patients with multi-

morbidity, renal impairment, or polypharmacy.¹³ Therefore, the present results should be viewed as preclinical support for the mechanistic rationale of multimodal therapy rather than direct clinical evidence.

Overall, the study demonstrates that D-Na has a moderate time-dependent antinociceptive effect in the AA model, while its combination with GAB or PREG provides a significantly stronger restoration of mechanical nociceptive threshold in the late phase of chronic inflammatory pain. The D-Na + AMI combination showed a moderate effect, but its superiority over monotherapy was not statistically definitive. These findings support the hypothesis that persistent arthritis-related hyperalgesia may require simultaneous targeting of peripheral inflammatory mechanisms and central pain amplification pathways.

Several limitations should be considered when interpreting these findings. The relatively small sample size and absence of formal a priori power calculation may reduce the statistical robustness of borderline results, particularly for the D-Na + AMI combination. Although randomisation and allocation concealment were applied, potential bias related to blinding during treatment administration and behavioural assessment cannot be fully excluded. In addition, the study included only male rats and relied on behavioural assessment of mechanical hyperalgesia without direct evaluation of inflammatory mediators, glial activation, spinal excitability, or molecular markers of central sensitisation. Therefore, while the obtained results support the mechanistic rationale for diclofenac-based multimodal analgesia, their translation to clinical arthritis-related pain should be made with caution.

Future studies should include larger sample sizes, formal power calculation, blinded outcome assessment, dose-response analysis and evaluation of molecular or histological markers related to neuroinflammation and central sensitisation. Such studies would help clarify whether the enhanced behavioural effect of diclofenac combined with gabapentinoids is directly associated with modulation of central pain mechanisms and whether these combinations may provide a basis for safer NSAID-sparing approaches in chronic inflammatory pain.

Conclusion

In the rat model of adjuvant arthritis, D-Na monotherapy provided only partial and time-dependent restoration of mechanical nociceptive threshold, whereas its combinations with gabapentin or pregabalin demonstrated significantly greater antinociceptive efficacy in the late phase of inflammatory pain. These findings suggest that persistent arthritis-related hyperalgesia is not fully controlled by peripheral anti-inflammatory mechanisms alone and may require additional modulation of centrally mediated pain amplification. The combination of diclofenac with amitriptyline showed a moderate effect that remained at the threshold of statistical significance, indicating potential therapeutic relevance but requiring further optimisation of dose, treatment duration and study power. Overall, the results support the rationale for diclofenac-based multimodal analgesia, particularly with gabapentinoids, as a promising approach for targeting both peripheral and central components of chronic inflammatory pain.

Ethics

The study was conducted in accordance with internationally accepted principles for the care and use of laboratory animals and complied with the ARRIVE 2.0 guidelines (2020), the European Directive 2010/63/EU and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). All procedures were performed in accordance with the Law of Ukraine "On Protection of Animals from Cruelty" (No 3447-IV).

The experimental protocol was reviewed and approved by the Commission on Ethics and Bioethics of the School of Medicine of VN Karazin Kharkiv National University (Protocol No 9, dated 6 May 2025). All efforts were made to minimise animal suffering and to reduce the number of animals used. Animals were maintained under standard laboratory conditions with free access to food and water. Euthanasia was performed in accordance with the AVMA Guidelines for the Euthanasia of Animals (2020).

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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