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*Original Article*

# Anti-Inflammatory Activity of a New Dipharmacophore Derivative of Propionic Acid

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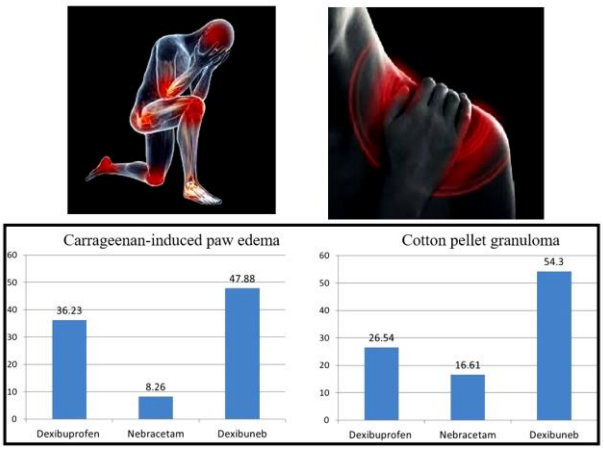
**ABSTRACT**

This experimental laboratory animals research aimed at the anti-inflammatory activity of a new dipharmacophore derivative dexibuprofen and nebracetam when modeling an acute and chronic inflammatory reaction. The studied dipharmacophore compound based on dexibuprofen and nebracetam is indicated by the dexibuneb laboratory code and has the chemical formula (2S) -N - ((1-benzyl-5-hydroxypyrrolidin-3-yl) methyl) -2- (4-isobutylphenyl) propanamide. Dexibuneb was administered intragastrically at a dose of 80 mg/kg; dexibuprofen and nebracetam were administered intragastrically at a dose of 40 mg/kg. The minimum increase in paw edema was recorded in the group of animals treated with dexibuneb. It amounted to 33.61 ± 7.41 of the initial value, which is statistically significantly ($p < 0.05$) less than the values both in the control group and in the groups treated with dexibuprofen nebracetam monotherapy. The use of dexibuneb reduced the increase in dry weight of the granuloma by 43.09% ($p < 0.05$) relative to the increase in the control group. In this study, when studying the severity of the anti-inflammatory effect on acute and chronic inflammation models, we found a positive pharmacodynamic interaction of molecular fragments of various therapeutic orientations, combined in an innovative dipharmacophore compound with the laboratory name dexibuneb. Inhibition of paw edema after introducing carrageenan into the plantar aponeurosis and the increase in the mass of granulomas implanted in rat armpits was statistically significantly more pronounced in the groups of animals treated with dexibuneb than with dexibuprofen and nebracetam monotherapy.

**GRAPHICAL ABSTRACT**

**Anti-Inflammatory Activity of a New Dipharmacophore Derivative of Propionic Acid**

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## Introduction

Pain and inflammation are a drain on productivity for farm animals. Many chemical mediators and receptor systems are involved in these phenomena, which can increase pain perception in terms of quality, quantity, time, and space. The nociceptive system's complexity and plasticity can make clinical pain control difficult [1, 2]. Understanding the structure and chemical signals associated with this mechanism will help increase the effectiveness of currently available analgesics and provide targets for the production of newer, more precise painkillers [3, 4]. There will still be circumstances on the farm where the introduction of discomfort or inflammation cannot be stopped, despite our best attempts to avoid illness, such as diseases and procedures performed on animals for management, such as castration and tail-docking [1,2]. However, timely or even pre-emptive care with sufficient anti-inflammatory or pain medication for these diseases or procedures will reap the rewards [5, 6]. As part of normal day-to-day operating procedures, a rigorous plan to minimize pain and inflammation must be enforced on the farm as a way of enhancing animal health and improving the economic performance of the farm [6–9]. Pain and inflammation are the physiological reactions of the body to damage to tissues or nerves, infection, and genetic changes [10,11]. Pain is a medical problem with high social significance, which is caused by a significant decrease in the quality of life of people suffering from pain, huge personal expenses of such patients, and a heavy burden on the healthcare system [12]. Performing a protective, adaptive function, pain during prolonged stimulation require a chronic course, exerting a maladaptive effect and acquiring pathogenic significance for the body [13]. Today, there is a close relationship between pain and inflammation. Pain is caused by all those pro-inflammatory agents that lead to hyperalgesia by activating the corresponding receptors expressed by nociceptive terminals [11]. The attention of practicing doctors and scientists all over the world has been riveted for many years to the problem of treating acute and chronic pain [14].

A significant contribution to the development of pain control belongs to analgesic and anti-inflammatory drugs; however, they are not always effective enough, and many of them even have an unfavorable safety profile. The success of pain treatment is limited due to our incomplete understanding of the molecular mechanisms underlying its transmission and perception. Therefore, a promising direction in the field of creating new drugs is the search for biologically active substances with anti-inflammatory and analgesic activity [15–21].

In the present study, we developed new dipharmacophore compounds based on substances with proven therapeutic activity, namely, the non-steroidal anti-inflammatory compound (NSAIDs), dexibuprofen, and the racetam compounds — nebracetam, which exhibits nootropic and cytoprotective effects, and also studied their anti-inflammatory effects on acute and chronic models of inflammation [22,23].

We accordingly studied the anti-inflammatory activity of a new dipharmacophore derivative dexibuprofen and nebracetam in modeling acute and chronic inflammation in the experiment on laboratory animals.

## Material and methods

The experimental study was conducted at the Research Institute of Pharmacology of Living Systems of Belgorod State National Research University. The study was performed in compliance with the requirements of General Requirements for the Competence of Testing and Calibration Laboratories 17025-2009, GOST R ISO 5725-2002, and the Rules of Laboratory Practice, approved by order of the Ministry of Healthcare and Social Development of the Russian Federation dated August 23rd, 2010 No. 708n.

Acute inflammation was modeled by local injection of 0.1 ml of carrageenan (1% in saline) into the plantar aponeurosis of the right hind paw of rats [24,25].

The studied dipharmacophore compound based on dexibuprofen and nebracetam is indicated by the dexibuneb laboratory code and has the chemical formula (2*S*)-*N*-((1-benzyl-5-hydroxypyrrolidin-3-yl)methyl)-2-(4-isobutylphenyl) propanamide. Dexibuneb was administered intragastrically 45 minutes before modeling inflammation. The dose of dexibuprofen and nebracetamwas was calculated considering conversion factors taking into account therapeutic doses for humans, amounting to 40 mg/kg for the rat. Because up to 2 active metabolites of dexibuprofen and nebracetam are metabolized in the body of dexibuneb in a ratio of 50/50, a dosage of 80 mg/kg is selected as the therapeutic dose of dexibuneb.

Animals were divided into the following experimental groups (n = 10):

Group 1- intact animals without modeling inflammation;

Group 2- control - carrageenan + carrier (1% starch solution at a dose of 1 ml / kg intragastrically);

Group 3- carrageenan + dexibuprofen (40 mg / kg);

Group 4- carrageenan + nebracetam 40 mg / kg;

Group 5 - Carrageenan + Dexibuneb 80 mg / kg

Foot volume was measured to the ankle joint oncometrically twice - immediately before the injection of carrageenan and then 4 hours after introducing the flagogen. The inhibitory effect was calculated by the (1):

$$\%\ \text{Inhibition} = \frac{(\Delta V_C - \Delta V_T) \times 100}{\Delta V_C} \qquad (1)$$

Where% Inhibition is the inhibitory effect, $\Delta V_C$ and $\Delta V_T$ are the average increases in the volume of the edematous foot in the control and experimental groups.

Anti-inflammatory activity of dexibuneb has also been studied in a chronic inflammation model - granulomas caused by implantation of cotton pellets in rats. The grouping of animals and the drugs administered are described in the section on the description of the experiment in the background of an acute inflammatory reaction caused by the injection of carrageenan. 45 minutes after the administration of the test compounds, the animals were anesthetized (chloral hydrate 200 mg/kg), and a sterile autoclaved cotton ball (made of bleached cotton) weighing 10 ± 1 mg each, saturated with normal saline, was implanted subcutaneously on both sides under the armpit. Animals were kept under aseptic conditions throughout the study. The administration of the test substances continued for another six days after pellet implantation. On the 8th day, cotton balls were excised from anesthetized animals, freeing them from foreign tissue. The recovered cotton balls were dried for 12 hours at 60 °C. The dried pellets were weighed, and the increase in their dry weight was evaluated. The inhibitory effect was calculated by the (2):

$$\%\ \text{Inhibition} = \frac{(\Delta M_C - \Delta M_T) \times 100}{\Delta M_C} \qquad (2)$$

Where % Inhibition Inhibitory Effect, $\Delta M_C$ and $\Delta M_T$ the average weight of pellets in the control and experimental groups [26,27].

**Result and Dissection**

Based on the results of the study, it was found that the introduction of carrageenan into the plantar aponeurosis of the control group of animals causes a pronounced inflammatory response and a statistically significant ($p < 0.05$), in comparison to intact animals, increase in foot volume. Thus, in intact animals, an increase in foot volume 4 hours after the administration of physiological saline was 2.87 ± 4.9%, and in the group of animals with an inflammation model, it was 64.02 ± 11.71% (Table 1). After the administration of the drugs nebracetam and dexibuprofen, the expected results were found - dexibuprofen (40 mg/kg) led to a statistically significant decrease in the growth of the foot volume against the background of the administration of carrageenan (40.29 ± 6.75%, $p < 0.05$ compared with the control). In contrast, nebracetam was not effective (59.22 ± 7.17%) (Table 1).

Next, we analyzed the increase in foot volume after modeling an acute inflammatory reaction in the group of animals treated with dexibuneb at a

dose of 80 mg/kg intragastrically (Table 1). In this group, the increase in foot volume was 33.61 ± 7.41 from the initial value, which is statistically significantly ($p < 0.05$) less than the values both in the control group and in the groups treated with dexibuprofen and nebracetam monotherapy (Table 1). When calculating the inhibitory effect, it was found that% inhibition of foot edema caused by the injection of carrageenan into the plantar aponeurosis also turned out to be maximal in the group of animals receiving the dipharmacophore compound of nebracetam and dexibuprofen (Figure 1).

**Table 1:** The results of a study of the anti-inflammatory activity of the dipharmacophore derivative dexibuprofen and nebracetam (Dexibuneb) in the modeling of acute inflammation by introducing carrageenan into the plantar aponeurosis (M ± m, n = 10)

| Groups | Initial foot volume (ml) | Foot volume after 4 hours (ml) | Foot mass gain (%) |
|---|---|---|---|
| Intact | 0.73±0.04 | 0.75±0.06 | 2.87±4.9 |
| Control | 0.74±0.04 | 1.21±0.07* | 64.02±11.71* |
| Dexibuprofen(40 mg/kg) | 0.75±0.05 | 1.05±0.08** | 40.29±6.75** |
| Nebracetam (40 mg/kg) | 0.73±0.01 | 1.17±0.06* | 59.22±7.17* |
| Dexibuneb (80 mg/kg) | 0.73±0.04 | 0.98±0.07**b | 33.61±7.41**ab |

Note: * - at $p < 0.05$ in comparison with intact animals; ** - at $p < 0.05$ in comparison with the control; a - at $p < 0.05$ in comparison with dexibuprofen; b - at $p < 0.05$ in comparison with nebracetam

When analyzing the anti-inflammatory effect in a model of chronic inflammation, we obtained similar experimental data. The use of dexibuprofen led to statistically significant inhibition of granuloma mass by 21.18% compared with the control ($p < 0.05$). Although nebracetam allowed to reduce the increase in granuloma mass by 12.77% relative to the control group; however, indicators of both relative granuloma mass and percent growth were not statistically significant (Table 2).

**Table 2:** The results of a study of the anti-inflammatory activity of the dipharmacophore derivative dexibuprofen and nebracetam (Dexibuneb) in modeling chronic inflammation during implantation of cotton pellets (M ± m, n = 10)

| Groups | Initial pellet mass (mg) | Pellet mass after 7 days (mg) | Dry weight gain (%) |
|---|---|---|---|
| Control | 19.94±1.26 | 35.90±3.9 | 80.09±16.21 |
| Dexibuprofen (40 mg/kg) | 19.87±1.02 | 31.6±2.3* | 58.91±5.86* |
| Nebracetam (40 mg/kg) | 19.73±1.03 | 33.04±3.21 | 67.32±11.29 |
| Dexibuneb (80 mg/kg) | 19.68±1.03 | 26.97±2.69*ab | 37.00±10.74*ab |

Note: * - at $p < 0.05$ in comparison with the control; a - at $p < 0.05$ in comparison with dexibuprofen; b - at $p < 0.05$ in comparison with nebracetam.

The lowest increase in granuloma mass, indicating a pronounced anti-inflammatory activity of the test compound, was found in the group treated with dexibuneb. The pellet mass 7 days after its implantation in this group of animals was statistically significantly ($p < 0.05$) lower than in all other experimental groups, including monotherapy with drugs. The use of dexibuneb led to a decrease in the increase in the dry mass of the granuloma by 43.09% (($p < 0.05$) relative to the increase in the control group (Table 2).

The inhibitory effect of dexibuneb calculated in the study on a model of chronic inflammation was 54.3% (Figure 1).

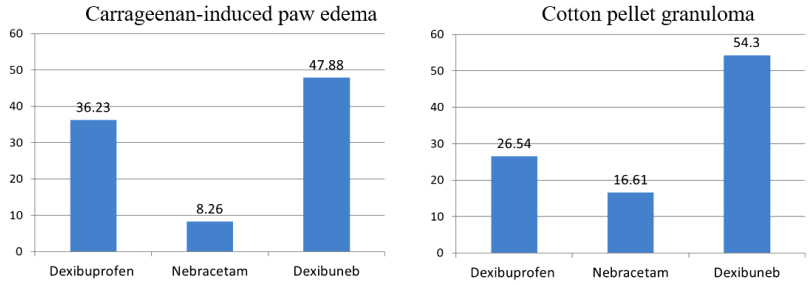


**Figure 1:** The inhibitory effect of the dipharmacophore derivative dexibuprofen and nebracetam (Dexibuneb) in modeling acute and chronic inflammation (% inhibition)

According to statistics from health services and leading experts on the problem of pain in many developed countries, millions of people suffer from chronic pain syndromes who change their physical and emotional state, reduce their quality of life, and reduce their ability to work [28–30]. Chronic pain is associated with significant violations of the level of social and labor adaptation. In most cases, chronic pain syndrome is accompanied by depression, causes negative emotional experiences, and reduces the patient's quality of life. According to WHO, pain syndromes are one of the leading causes (from 11.3 to 40%) of visits to a doctor in the primary health care system [31]. In the structure of neurological admission, patients with chronic pain syndromes (CHD) account for up to 52.5% [32].

We believe that the search for new pharmacological agents for the treatment of pain should be carried out in the field of innovative dipharmacophore compounds having fragments with different pathogenetic; therefore, therapeutic orientations. NSAIDs are widely used in the medical treatment of various diseases accompanied by pain or inflammation. Their widespread prevalence was ensured by the absence of side effects inherent in opiates: sedation, respiratory depression, and addiction. NSAIDs can suppress inflammation, lower body temperature, and reduce pain intensity. Nootropics (neurometabolic stimulants) are drugs designed to provide specific effects on higher mental functions. We previously studied the pharmacological activity of a number of nootropics, cytoprotection, non-steroidal anti-inflammatory drugs [32,33].

Here, we studied the anti-inflammatory activity on the models of acute and chronic inflammation of the new dipharmacophore compound developed in this study based on substances with proven therapeutic activity - dexibuprofen and a compound from the racetam group - nebracetam. The study showed that the di pharmacophore compound of dexibuprofen and nebracetam with the laboratory name dexibuneb has powerful anti-inflammatory activity, both in the acute and chronic inflammation models.

The rat paw edema model induced by carrageenan is a well-known model for evaluating the anti-inflammatory effects of drugs [34]. This model shows that the anti-inflammatory effect of dexibuneb was statistically significant ($p < 0.05$), 6.68% higher than the anti-inflammatory effect of dexibuprofen monotherapy and 25.61% ($p < 0.05$) higher than in the group animals receiving nebracetam. A similar tendency was found in the model of chronic inflammation - the use of dexibuneb led to the most effective reduction in the increase in the dry mass of the granuloma

In the analysis of the calculated inhibitory effect, we found that the maximum percentage of inhibition of acute foot edema caused by carrageenan and cotton pellet implantation was found in the group of animals treated with dipharmacophoredexibuneb.

Thus, we confirmed our hypothesis about increasing the effectiveness of non-steroidal anti-inflammatory drugs by introducing into the molecule a new active center acting on a pathogenetic point different from the point of application of NSAIDs. The mechanism of the detected action of dexibuprofen is associated

with its main mechanism of action - inhibition of COX-2. We suggest that the mechanism of the anti-inflammatory effect of the drug nebracetam is associated primarily with the manifestation of its antihypoxic and cytoprotective properties and the ability to inhibit the synthesis of nitric oxide, as one of the factors that are toxic when an inflammatory reaction occurs.

## Conclusion

In this study, when studying the severity of the anti-inflammatory effect on models of acute and chronic inflammation, we found a positive pharmacodynamic interaction of molecular fragments of various therapeutic orientations, combined in an innovative dipharmacophore compound with the laboratory name dexibuneb. Inhibition of paw edema after the introduction of carrageenan into the plantar aponeurosis and the increase in the mass of granulomas implanted in rat armpits was statistically significantly more pronounced in the groups of animals treated with dexibuneb than with dexibuprofen and nebracetam monotherapy.

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## Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

## Conflict of Interest

We have no conflicts of interest to disclose.

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