УДК 615.212/.214:577.175.853
https://doi.org/10.20538/1682-0363-2023-2-6-13

Analgesic effect of a bradykinin antagonist – a 1,4-benzodiazepine-2-one derivative

Aliforenko A.E.[1, 2], Bykov V.V.[1, 2], Bykova A.V.[2], Motov V.S.[2], Stankevich S.A.[2], Pavlovsky V.I.[2, 3], Khazanov V.A.[2], Vengerovskii A.I.[1]

[1] *Siberian State Medical University*
2, Moscow Trakt, Tomsk, 634050, Russian Federation

[2] *Innovative Pharmacology Research LLC (IPHAR LLC)*
79/4, Elizarovykh Str., Tomsk, 634021, Russian Federation

[3] *National Research Tomsk Polytechnic University (TPU)*
30, Lenina Av., Tomsk, 634050, Russian Federation

ABSTRACT

Aim. To study the analgesic effect of a new 1,4-benzodiazepine-2-one derivative (codenamed PAV-0056) in pain models in mice, its anti-inflammatory effect in experimental exudative inflammation in rats, and its potential ulcerogenic effect.

Materials and methods. A 1,4-benzodiazepine-2-one derivative (codenamed PAV-0056) was orally administered in polyvinylpyrrolidone (PVP) solution to 192 CD-1 mice weighing 20–25 g and 140 Sprague – Dawley rats weighing 250–300 g. The analgesic effect of the PAV-0056 compound at a dose of 0.01, 0.1, and 1 mg / kg was studied in murine acute thermal pain models (hot plate test, hot water immersion tail-flick test), acute chemogenic pain models (formalin test), and visceral spasticity-related pain models (acetic acid-induced writhing test). The anti-inflammatory effect of PAV-0056 at doses of 0.01, 0.1, and 1 mg / kg was studied in an experimental rat model of inflammation induced by subplantar administration of bradykinin and histamine. The potential ulcerogenic effect was studied in intact rats, who were injected with PAV-0056 at doses of 1 and 50 mg / kg four times. The analgesic effect of the PAV-0056 compound was compared to that of diclofenac sodium at a dose of 10 mg / kg and tramadol at a dose of 20 mg / kg. Its anti-inflammatory and potential ulcerogenic effects were compared to those of diclofenac sodium at a dose of 10 mg / kg.

Results. In the hot plate test, the PAV-0056 compound at a dose of 0.1 mg / kg increased response latency in mice by 36%, and at a dose of 1 mg / kg, it increased response latency by 46% ($p < 0.05$). In the tail-flick test, the PAV-0056 compound at a dose of 1 mg / kg increased response latency to heat stimulation in mice by 46% ($p < 0.05$). After subplantar administration of formalin, PAV-0056 at doses of 0.01–1 mg / kg had a pronounced analgesic effect, as shown by a decrease in the number of pain responses by 39–55% ($p < 0.05$). When mice were intraperitoneally injected with an acetic acid solution, the PAV-0056 compound at doses of 0.1 and 1 mg / kg reduced the frequency of writhings by 46 and 57%, respectively; at a dose of 0.1 mg / kg, it delayed the onset of the first writhing by 21% ($p < 0.05$). In experiments on rats, the PAV-0056 compound prevented the development of exudative inflammation induced by subplantar administration of bradykinin and did not have an anti-inflammatory effect in histamine-induced inflammation. PAV-0056 did not cause formation of gastric ulcers and gastric mucosal bleeding.

Conclusion. A 1,4-benzodiazepine-2-one derivative, PAV-0056, has a pronounced analgesic effect in models of thermal, chemogenic, somatic, and visceral pain in a wide range of doses (0.01–1 mg / kg). Its analgesic effects are the same as those of diclofenac sodium at a dose of 10 mg / kg and tramadol at a dose of 20 mg / kg. The analgesic effect of the PAV-0056 compound is selective, depends little on suppression of inflammatory exudation, and is caused by bradykinin antagonism. This substance has low toxicity and does not damage the gastric mucosa.

Keywords: 1,4-benzodiazepine-2-one derivative, analgesic, anti-inflammatory, and potential ulcerogenic effects, mice, rats

✉ *Bykov Vladimir V.,* preclin5_dep@iphar.ru

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

Source of financing. The study was carried out with the financial support of Organica JSC (Novokuznetsk).

Conformity with the principles of ethics. The study was approved by the local Ethics Committee at IPHAR LLC (Protocol No. 77/2020 of 24.11.2020) and Siberian State Medical University (Protocol No. 8992 of 21.02.2022).

For citation: Aliforenko A.E., Bykov V.V., Bykova A.V., Motov V.S., Stankevich S.A., Pavlovsky V.I., Khazanov V.A., Vengerovskii A.I. Analgesic effect of a bradykinin antagonist – a 1,4-benzodiazepine-2-one derivative. *Bulletin of Siberian Medicine.* 2023;22(2):6–13. https://doi.org/10.20538/1682-0363-2023-2-6-13.

Анальгетическая активность антагониста брадикинина – производного 1,4-бензодиазепин-2-она

Алифоренко А.Е.[1, 2], Быков В.В.[1, 2], Быкова А.В.[2], Мотов В.С.[2], Станкевич С.А.[2], Павловский В.И.[2, 3], Хазанов В.А.[2], Венгеровский А.И.[1]

[1] Сибирский государственный медицинский университет (СибГМУ)
Россия, 634050, г. Томск, Московский тракт, 2

[2] ООО «Инновационные Фармакологические Разработки» (ООО «Ифар»)
Россия, 634021, г. Томск, ул. Елизаровых, 79/4

[3] Национальный исследовательский Томский политехнический университет (НИ ТПУ)
Россия, 634050, г. Томск, пр. Ленина, 30

РЕЗЮМЕ

Цель – изучить анальгетическую активность нового производного 1,4-бензодиазепин-2-она (шифр – PAV-0056) на моделях боли у мышей, противовоспалительное действие при экспериментальном экссудативном воспалении у крыс, а также его потенциальное ульцерогенное действие.

Материалы и методы. Производное 1,4-бензодиазепин-2-она, обозначенное шифром PAV-0056, вводили в растворе поливинилпирролидона в желудок 192 мышам стока CD-1 массой тела 20–25 г и 140 крысам стока Sprague Dawley массой тела 250–300 г. Анальгетическую активность соединения PAV-0056 в дозах 0,01; 0,1 и 1 мг/кг изучали у мышей на моделях острой термической боли (тесты «горячая пластина» и отдергивания хвоста при погружении в горячую воду), острой хемогенной боли (формалиновый тест) и висцеральной спастической боли (тест «уксусные корчи»). Противовоспалительное действие PAV-0056 в дозах 0,01; 0,1 и 1 мг/кг исследовали при экспериментальном воспалении, вызванном у крыс субплантарным введением брадикинина и гистамина. Потенциальное ульцерогенное влияние изучали у интактных крыс, которым вещество PAV-0056 в дозах 1 и 50 мг/кг вводили 4 раза. Анальгетический эффект соединения PAV-0056 сравнивали с действием диклофенака натрия в дозе 10 мг/кг и трамадола в дозе 20 мг/кг, противовоспалительное и возможное ульцерогенное действие – с влиянием диклофенака натрия в дозе 10 мг/кг.

Результаты. В тесте «горячая пластина» соединение PAV-0056 в дозе 0,1 мг/кг увеличивало у мышей время до появления первой болевой реакции на 36%, в дозе 1 мг/кг – на 46% ($p < 0,05$). В тесте отдергивания хвоста при погружении в горячую воду соединение PAV-0056 в дозе 1 мг/кг увеличивало латентное время наступления термической боли у мышей на 46% ($p < 0,05$). При субплантарном введении формалина соединение PAV-0056 в дозах 0,01–1 мг/кг оказывало выраженное анальгетическое действие, что проявлялось уменьшением на 39–55% количества болевых реакций ($p < 0,05$). При внутрибрюшинной инъекции мышам раствора уксусной кислоты соединение PAV-0056 в дозах 0,1 и 1 мг/кг уменьшало количество «корчей» на 46 и 57% соответственно, в дозе 0,1 мг/кг отдвигало наступление первой «корчи» на 21% ($p < 0,05$). В экспериментах на крысах соединение PAV-0056 препятствовало развитию экссудативного воспаления, вызванного субплантарным введением брадикинина, и не оказывало противовоспалительного эффекта при гистаминовом воспалении, не вызывало образования язв и кровоизлияний на слизистой оболочке желудка.

Заключение. Производное 1,4-бензодиазепин-2-она PAV-0056 в широком диапазоне доз (0,01–1 мг/кг) вызывает выраженную анальгезию на моделях термической, хемогенной, соматической и висцеральной боли, по анальгетической активности не уступает эффекту диклофенака натрия в дозе 10 мг/кг и трамадола в дозе 20 мг/кг. Анальгетическое действие соединения PAV-0056 является селективным, мало зависит

от подавления воспалительной экссудации и обусловлено антагонизмом с брадикинином. Это вещество малотоксично и не повреждает слизистую оболочку желудка.

Ключевые слова: производное 1,4-бензодиазепин-2-она, анальгетическое, противовоспалительное и потенциальное ульцерогенное действие, мыши, крысы

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Исследование выполнено при финансовой поддержке АО «Органика» (г. Новокузнецк).

Соответствие принципам этики. Исследование одобрено локальными этическими комитетами ООО «Инновационные Фармакологические Разработки» (протокол № 77/2020 от 24.11.2020) и СибГМУ (протокол № 8992 от 21.02.2022).

Для цитирования: Алифоренко А.Е., Быков В.В., Быкова А.В., Мотов В.С., Станкевич С.А., Павловский В.И., Хазанов В.А., Венгеровский А.И. Анальгетическая активность антагониста брадикинина – производного 1,4-бензодиазепин-2-она. *Бюллетень сибирской медицины.* 2023;22(2):6–13. https://doi.org/10.20538/1682-0363-2023-2-6-13.

INTRODUCTION

Pain is a complex psycho-emotional sensation in response to the effect of nociceptive stimuli, characterized by impaired adaptation and deterioration in patients' quality of life [1]. To alleviate pain, opioid and non-opioid analgesics, as well as non-steroidal anti-inflammatory drugs (NSAIDs) are used. Opioid analgesics eliminate severe pain of any etiology, but depress the respiratory center and generate a risk of physical and psychological dependence and addiction [2]. NSAIDs are most effective for nociceptive pain at the inflammation site; their side effects include ulcerogenic and nephrotoxic effects, cardiovascular diseases, bronchospasm, and bleeding [3].

One of the modern strategies in the search for new analgesics is development of autacoid inhibitors (bradykinin, histamine, serotonin), which prevent their activating effects on the nociceptive system [4–6]. The nonapeptide bradykinin is generated during inflammation, allergies, and infections via cleavage of kininogen by the action of kallikrein and activates metabotropic B receptors [7]. Bradykinin directly irritates sensitive nerve endings and promotes secretion of other algogenic factors, such as substance P, neurokinin A, and calcitonin gene-related peptide. At the inflammation site, bradykinin dilates capillaries, disrupts cell – cell contacts, and increases exudation and migration of neutrophils, lymphocytes, and macrophages [8].

In clinical practice, there are no analgesics with anti-bradykinin action. Bradykinin B receptor is blocked by 1,4-benzodiazepine-2-one derivatives [9–12]; among them, the compound PAV-0056 has the greatest analgesic effect [13].

The aim of this work was to study the analgesic effect of a new 1,4-benzodiazepine-2-one derivative PAV-0056 in experimental models of pain and inflammation, as well as to evaluate its potential ulcerogenic effect.

MATERIALS AND METHODS

The PAV-0056 compound is methyl-2-(7-nitro-2-oxo-5-phenyl-3-propoxy-2,3-dihydro-1*H*-benzo[e][1,4]diazepine-1-yl)acetate (Fig.1). Derivatives of 1,4-benzodiazepine-2-ones were synthesized at IPHAR LLC based on molecular design and study of the relationship between the chemical structure and affinity to benzodiazepine receptors and analgesic effects [14]. Among this group of substances, the PAV-0056 compound has minimal toxicity, and its oral LD50 in mice and rats is over 2,000 mg / kg.

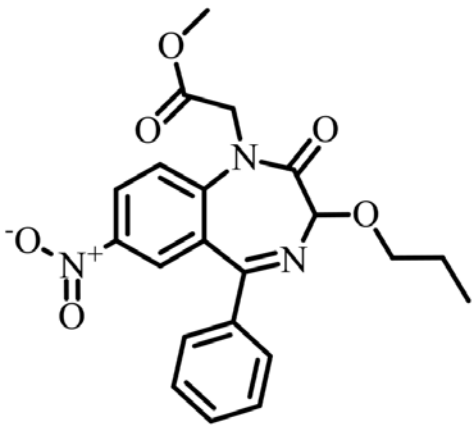

Fig. 1. Structural formula of the PAV-0056 compound

The experiments were carried out at the R&D center of IPHAR LLC in 192 pathogen-free male CD-1 mice weighing 20–25 g and 140 male Sprague – Dawley rats weighing 250–300 g. The animals were obtained from the Department of Laboratory Animals of the R&D center. They were kept in groups of 5–6 individuals each in plastic cages at 20–23 °C, relative humidity of no more than 50%, exhaust – supply ratio of 8 :10, in a 12 : 12 light /dark cycle.

The study was approved by the local Ethics Committees at IPHAR LLC (Protocol No. 77/2020 of 24.11.2020) and Siberian State Medical University (Protocol No. 8992 of 21.02.2022). It was conducted in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the principles and rules of Good Laboratory Practice (Decision of the Council of Eurasian Economic Commission No. 81 of 03.11.2016 and the National Standard of the Russian Federation (GOST) 33044-2014).

The PAV-0056 compound at doses of 0.01, 0.1, and 1 mg / kg or the reference listed drugs diclofenac sodium (Hemofarm, Serbia) at a dose of 10 mg / kg [15] and tramadol (Pharmaceuticals Formenti S.p.A., Italy) at a dose of 20 mg / kg [16] were orally administered to mice to assess the analgesic effect. All substances were dissolved in 0.5 ml of 1% aqueous solution of polyvinylpyrrolidone (PVP) (Plasdone S-630, Ashland Inc., USA). Control animals received the vehicle in an equivalent volume. Doses of the PAV-0056 compound were selected based on previous pilot studies. Analgesic effects were evaluated 60 min after the administration of the PAV-0056 compound, diclofenac sodium or tramadol, and compared with those in control animals. Each experimental group contained 6–10 animals.

Hot plate test. Mice (6 groups, n = 6 animals per group) were placed on a metal surface heated to a temperature of 55 ± 1 °C (thermal table HWT-75, Russia). We determined response latency, measured by paw withdrawal and licking. The mice were kept on the hot plate for no more than 1 min to avoid injury [4, 17].

Tail-flick test. The tails of mice (6 groups, n = 10 animals per group) were half immersed in water at a temperature of 45 ± 1 °C (Sakura 1450 water bath, Japan). We measured the time before the tail flicked to the side [4, 17].

Formalin test. Mice (6 groups, n = 10 animals per group) were injected with 0.02 ml of 0.5% formalin (Sigma-Aldrich, USA) into the plantar aponeurosis of the hind limb. A pain response was registered by the number of licks and shakes of the injured paw during 60 min. In the first 15 min (phase I), acute phase pain developed due to sensitization of peripheral structures of the nociceptive system, in the next 45 min (phase II), tonic pain developed, which was caused by activation of central mechanisms of the nociceptive system [17, 18].

Acetic acid-induced writhing test. Mice (6 groups, n = 6 animals per group) were intraperitoneally injected with 0.75% aqueous acetic acid solution (Sigma-Aldrich, USA) in a volume of 0.1 ml per 10 g of body weight. We assessed the number of abdominal muscle contractions (writhings) and time to the onset of the first writhing within 20 min [17, 18].

The anti-inflammatory effect of the PAV-0056 compound was studied in models of acute exudative inflammation. Rats (10 groups, n = 10 animals per group) were injected with 0.1 ml of 0.1% aqueous bradykinin solution (Sigma-Aldrich, USA) or 2% aqueous histamine solution (Sigma-Aldrich, USA) into the aponeurosis of the hind limb. The other hind limb of these animals was injected with 0.1 ml of sodium chloride (control). The PAV-0056 compound at doses of 0.01, 0.1, and 1 mg / kg or diclofenac sodium at a dose of 10 mg / kg [19] was injected 1 h before the injection of bradykinin or histamine. The limb volume was measured using a plethysmometer (UGO BASIL, Italy) 30 min after the injection of inflammatory mediators. The degree of edema reduction in the inflamed limb was expressed as a percentage of the control [4].

To study the potential ulcerogenic effect, intact rats (4 groups, n = 10 animals per group) were orally administered the PAV-0056 compound at doses of 1 and 50 mg / kg or diclofenac sodium at a dose of 10 mg / kg four times (with an interval of 24 h). The animals were withdrawn from the experiment in a CO_2 atmosphere 3 h after the last administration. The gastric mucosa was studied for erosions, ulcers, and hemorrhages using a stereoscopic microscope (Observational Instruments, Russia) at 10× magnification. We assessed the degree of damage according to a four-point scale: 0 – no damage; 0.5 – mucosal hyperemia; 1 – 1 or 2 punctate hemorrhages on the mucous membrane; 2 – single erosion and punctate hemorrhages on the mucous membrane; 3 – multiple erosions and hemorrhages on the mucous membrane; 4 – massive hemorrhages and ulcers throughout the mucous membrane [4].

The results were statistically processed using the Statistica v. 8.0 (StatSoft, USA) software package. The normality of trait distribution was assessed using the Shapiro – Wilk test. The data were presented as $M \pm m$, where M is the mean and m is the standard error of the mean. The differences between the groups were identified by the Student's t-test and considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

In the hot plate test, the 1,4-benzodiazepine-2-one derivative PAV-0056 at a dose of 0.1 mg / kg prolonged the response latency by 36 %, at a dose of 1 mg / kg – by 46 % compared to that in mice treated with PVP ($p < 0.05$). PAV-0056 had no analgesic effect at a dose of 0.01 mg / kg. Diclofenac sodium at a dose of 10 mg / kg and tramadol at a dose of 20 mg / kg increased the response latency by 64 and 82%, respectively ($p < 0.05$) (Fig. 2). The analgesic effect of PAV-0056 at a dose of 0.1 and 1 mg / kg was comparable to that of diclofenac sodium at a dose of 10 mg / kg ($p > 0.05$), but was not as great as that of tramadol at a dose of 20 mg / kg ($p < 0.05$).

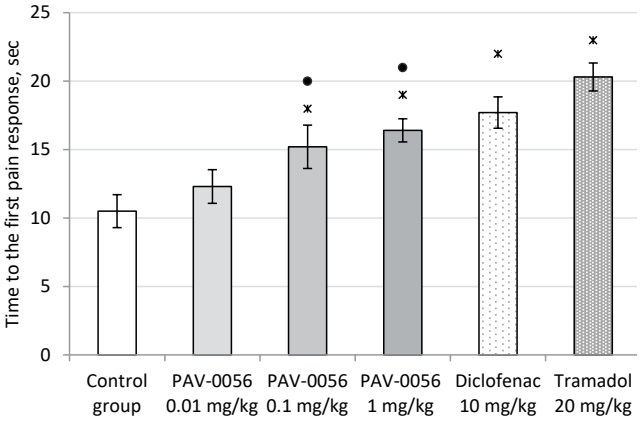

Fig. 2. Response latency in the hot plate test after the administration of PAV-0056, diclofenac sodium, and tramadol to mice: * differences with the control group, $p < 0.05$, • differences with the tramadol group, $p < 0.05$

PAV-0056 at a dose of 1 mg / kg prolonged the tail-flick latency by 46% in mice ($p < 0.05$), although it did not have an analgesic effect at doses of 0.1 and 0.01 mg / kg. Diclofenac sodium at a dose of 10 mg / kg increased the response latency by 46%, and tramadol at a dose of 20 mg / kg increased this parameter by 36%. The analgesic effect of tramadol was weak: the response latency did not differ from that in mice treated with PVP ($p > 0.05$) (Fig. 3).

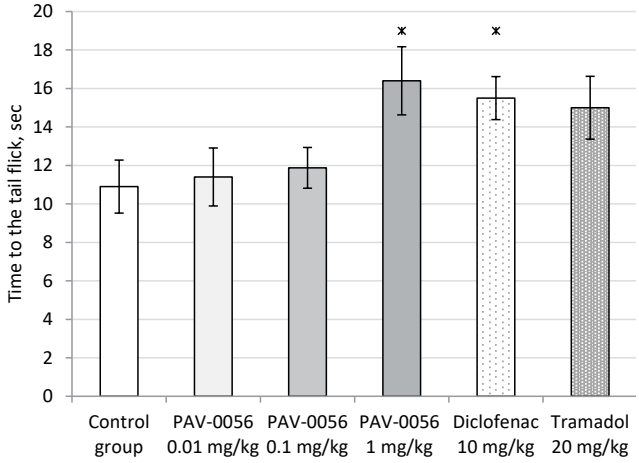

Fig. 3. Response latency in mice in the hot water immersion tail-flick test after the administration of PAV-0056, diclofenac sodium, and tramadol: * differences with the control group, $p < 0.05$

With subplantar formalin injection, PAV-0056 at a dose of 0.01–1 mg / kg reduced the number of pain responses in the acute and tonic pain phases ($p < 0.05$). Diclofenac sodium at a dose of 10 mg / kg and tramadol at a dose of 20 mg / kg also had an analgesic effect in both phases ($p < 0.05$). PAV-0056 at all studied doses had the same analgesic effects as diclofenac sodium and tramadol (Table 1).

PAV-0056 at a dose of 0.1 mg / kg reduced the frequency of writhings caused by the intraperitoneal injection of acetic acid by 46% and at a dose of 1 mg / kg – by 57%. At a dose of 0.1 mg / kg, it delayed the onset of the first writhing by 21% ($p < 0.05$). Diclofenac sodium and tramadol reduced the frequency of writhings by 49 and 68%, respectively and delayed the onset of the first writhing by 49 and 52%, respectively ($p < 0.05$). The analgesic effect of PAV-0056 at a dose of 0.1 and 1 mg / kg was the same as that of diclofenac sodium at a dose of 10 mg / kg; at a dose of 0.1 mg / kg, it was weaker than that of tramadol at a dose of 20 mg / kg (Table 2).

The contribution of the anti-inflammatory effect to the mechanism of the analgesic effect of PAV-0056 at doses of 0.01, 0.1, and 1 mg / kg was studied in rat models of exudative inflammation induced by the subplantar injection of bradykinin or histamine. The reference listed drug was diclofenac sodium at a dose of 10 mg / kg [19]. PAV-0056, administered 1 h before the injection of bradykinin, reduced limb edema by 33–58% ($p < 0.05$) (Table 3). The anti-inflammatory effect of PAV-0056 in histamine-induced edema was not significantly pronounced,

the volume of the limb decreased only by 6–16 % ($p > 0.05$). Diclofenac sodium prevented the development of paw edema by 43–54% in both models of inflammation ($p < 0.05$) (Table 4). The results of this experiment indicate bradykinin antagonism of PAV-0056 and a weak anti-histamine effect.

Table 1

Analgesic effect of PAV-0056, diclofenac sodium, and tramadol after administration to mice in the formalin test, *M* ± *m*

Number of responses	Control group, *n* = 10	PAV-0056 0.01 mg / kg, *n* = 10	PAV-0056 0.1 mg / kg, *n* = 10	PAV-0056 1 mg / kg, *n* = 10	Diclofenac 10 mg / kg, *n* = 10	Tramadol 20 mg / kg, *n* = 10
Phase I	31 ± 3	19 ± 1*	14 ± 1*	16 ± 1*	20 ± 4*	21 ± 3*
Phase II	15 ± 2	6 ± 1*	6 ± 2*	8 ± 3*#	7 ± 2*	9 ± 2*

* $p < 0.05$ compared to the controls (here and in Tables 2–4).
# One outlier was identified by the Smirnov – Grubbs test.

Table 2

Analgesic effect of PAV-0056, diclofenac sodium, and tramadol after administration to mice in the acetic acid-induced writhing test, *M* ± *m*

Parameter	Control group, *n* = 6	PAV-0056 0.01 mg / kg, *n* = 6	PAV-0056 0.1 mg / kg, *n* = 6	PAV-0056 1 mg / kg, *n* = 6	Diclofenac 10 mg / kg, *n* = 6	Tramadol 20 mg / kg, *n* = 6
Time before the first writhing, sec	258 ± 8	336 ± 66	312 ± 10*#	326 ± 59	385 ± 39*	393 ± 47*
Number of writhings	37 ± 1	29 ± 4	20 ± 2*	16 ± 2*	19 ± 4*	12 ± 3*

# $p < 0.05$ compared to the tramadol group.

Table 3

Anti-inflammatory effects of PAV-0056 and diclofenac sodium in the test with the subplantar injection of bradykinin in rats, *M* ± *m*

Parameter	Control group, *n* = 10	PAV-0056 0.01 mg / kg, *n* = 10	PAV-0056 0.1 mg / kg, *n* = 10	PAV-0056 1 mg / kg, *n* = 10	Diclofenac 10 mg / kg, *n* = 10
Volume of limb edema, ml	0.24 ± 0.02	0.16 ± 0.03*	0.08 ± 0.03*	0.10 ± 0.02*	0.11 ± 0.02*

Table 4

Anti-inflammatory effects of PAV-0056 and diclofenac sodium in the test with the subplantar injection of histamine in rats, *M* ± *m*

Parameter	Control group, *n* = 10	PAV-0056 0.01 mg / kg, *n* = 10	PAV-0056 0.1 mg / kg, *n* = 10	PAV-0056 1 mg / kg, *n* = 10	Diclofenac 10 mg / kg, *n* = 10
Volume of limb edema, ml	0.30 ± 0.8	0.32 ± 0.09	0.28 ± 0.11	0.25 ± 0.10	0.17 ± 0.05*

The 1,4-benzodiazepine-2-one derivative PAV-0056 at doses of 1 and 50 mg / kg, administered to the rats orally four times, did not have an ulcerogenic effect: there were no ulcers and hemorrhages in the gastric mucosa. Diclofenac sodium, administered four times at a dose of 10 mg / kg, caused multiple injuries, such as bleeding erosions and ulcers (3.5 points).

Thus, the 1,4-benzodiazepine-2-one derivative PAV-0056 exhibited analgesic effects in a wide dose range (0.01–1 mg / kg) which were the same as those of diclofenac sodium at a dose of 10 mg / kg and tramadol at a dose of 20 mg / kg. In the hot plate test, the PAV-0056 compound exerts a moderate analgesic effect because thermal somatic pain occurs with minimal involvement of inflammatory mediators, and thermoreceptors transmit pain information to the reticular formation in the midbrain and thalamus. In the tail-flick test, high-threshold mechanoreceptors are activated on a large surface of the thermal stimulation area; the pain signal through the polymodal C- and Aδ-fibers is carried mainly to the posterior horns of the spinal cord and then transmitted to the motor neurons of the anterior horns [20]. It can be assumed that the more pronounced analgesic effect of PAV-0056 in the hot plate test is associated with the activation of the antinociceptive system of the brain.

The PAV-0056 compound effectively alleviated chemogenic pain caused by formalin and acetic acid. Irritation of nociceptors at the damage site by bradykinin is of primary importance in the generation

of such pain. With the subplantar injection of formalin into the paw of the mice, PAV-0056 decreased the number of paw shakes more than the number of paw licks. This is due to the blockade of nociceptors and inhibition of the transmission of action potentials along afferent nerve fibers to the posterior horns of the spinal cord with suppression of the somatic reflex. The PAV-0056 compound has a weaker effect on the processing of pain signals in the cerebral cortex, so it interferes less with the formation of a conscious response to pain in the form of paw licking.

The PAV-0056 compound has analgesic and anti-inflammatory effects due to antagonism with bradykinin and is less effective in histamine-induced inflammation. The analgesic and anti-inflammatory effects of diclofenac sodium are caused by antagonism with prostaglandins, histamine and, to a lesser extent, with bradykinin. In a radioligand binding assay with Chinese hamster ovarian cytoreceptors, it was shown that some benzodiazepine derivatives, structurally similar to PAV-0056, block bradykinin B receptors at nanomolar concentrations [10].

An important advantage of the 1,4-benzodiazepine-2-one derivative PAV-0056 is the absence of an ulcerogenic effect. It is known that NSAIDs inhibiting cyclooxygenase-1 inhibit synthesis of gastroprotective prostaglandins and seriously damage the gastric mucosa [21]. In our experiment, diclofenac sodium resulted in the formation of numerous ulcers in the stomach, complicated by bleeding.

CONCLUSION

In the present study, the analgesic effect of a new 1,4-benzodiazepine-2-one derivative PAV-0056 was demonstrated for the first time in experimental models of thermal and chemogenic (somatic, visceral) pain. The PAV-0056 compound has a moderate anti-exudative effect in experimental inflammation induced by bradykinin. It does not damage the gastric mucosa. The analgesic effect of PAV-0056 is not weaker than that of diclofenac sodium and tramadol. The 1,4-benzodiazepine-2-one derivative is evaluated as a promising and safe drug with a selective analgesic effect.

REFERENCES

1. Kumar K.H., Elavarasi P., David C.M. Definition of pain and classification of pain disorders. *JCRI*. 2016;3:87–90. DOI: 10.15713/ ins.jcri.112.
2. Rizzi A., Ruzza C., Bianco S., Trapella C., Calo' G. Antinociceptive action of NOP and opioid receptor agonists in the mouse orofacial formalin test. *Peptides.* 2017;94:71–77. DOI: 10.1016/j.peptides.2017.07.002.
3. Lanas A., Chan F.K.L. Peptic ulcer disease. *Lancet.* 2017;390(10094):613–624. DOI: 10.1016/S0140-6736(16) 32404-7.
4. Mironov A.N. Guidelines on preclinical drug research. M.: Grif and K, 2013:944 (in Russ.).
5. Gonçalves E.C.D., Vieira G., Gonçalves T.R., Simões R.R., Brusco I., Oliveira S.M. et al. Bradykinin receptors play a critical role in the chronic postischaemia pain model. *Cell Mol. Neurobiol*. 2021;41(1):63–78. DOI: 10.1007/s10571-020-00832-3.
6. Litvitsky P.F. Inflammation. *Current Pediatrics.* 2006;5(4):75–81 (in Russ.).
7. Kaplan A.P., Joseph K., Silverberg M. Pathways for bradykinin formation and inflammatory disease. *J. Allergy Clin. Immunol.* 2002;109(2):195–209. DOI: 10.1067/mai.2002.121316.
8. Golias Ch., Charalabopoulos A., Stagikas D., Charalabopoulos K., Batistatou A. The kinin system – bradykinin: biological effects and clinical implications. Multiple role of the kinin system – bradykinin. *Hyppokratia*. 2007;11(3):124–128.
9. Dziadulewicz E.K., Brown M.C., Dunstan A.R., Lee W., Said N.B., Garratt P.J. The design of non-peptide human bradykinin B_2 receptor antagonists employing the benzodiazepine peptidomimetic scaffold. *Bioorg. Med. Chem. Lett.* 1999;9(3):463–468. DOI: 10.1016/s0960-894x(99)00015-3.
10. Wood M.R., Kim J.J., Han W., Dorsey B.D., Homnick C.F., DiPardo R.M. et al. Benzodiazepines as potent and selective bradykinin B1 antagonists. *J. Med. Chem.* 2003;46(10):1803–1806. DOI: 10.1021/jm034020y.
11. Pavlovsky V.I., Tsymbalyuk O.V., Martynyuk V.S., Kabanova T.A., Semenishyna E.A., Khalimova E.I. et al. Analgesic effects of 3-substituted derivatives of 1,4-benzodiazepines and their possible mechanisms. *Neurophysiology*. 2013;45:427–432. DOI: 10.1007/s11062-013-9389-y.
12. Pavlovsky V.I., Ushakov I.Yu., Kabanova T.A., Khalimova E.I., Kravtsov V.Kh, Andronati S.A. Synthesis and analgesic activity of 3-arylamino-1,2-dihydro-3н-1,4-benzodiazepin-2-ones. *Chemical and Pharmaceutical Journal*. 2015;49(9):22–27 (in Russ.). DOI: 10.30906/0023-1134-2015-49-9-22-27.
13. Pavlovsky V.I., Khazanov V.A., Stankevich S.A., inventors; Limited liability company "Innovative Pharmaceutical Developments" (IPHAR LLC), assignee. Derivatives of 1,4-benzodiazepin-2-one and their application. Russian Federation patent RU 2701557 C2. September 30, 2019. Russian Federation (in Russ.).
14. Virych P.A., Shelyuk O.V., Kabanova T.A., Khalimova E.I., Martynyuk V.S., Pavlovsky V.I. et al. Effect of 3-substituted 1,4-benzodiazepin-2-ones on bradykinin-induced smooth muscle contraction. *Ukr. Biochem. J.* 2017;89(1):31–37. DOI: 10.15407/ubj89.01.031.
15. Hasani A., Soljakova M., Jakupi M., Ustalar-Ozgen S. Preemptive analgesic effects of midazolam and diclofenac in rat model. *Bosn. J. Basic Med. Sci*. 2011;11(2):113–118. DOI: 10.17305/bjbms.2011.2593.
16. Bandapati S., Podila K.S., Yadala V.R. Comparative study of antinociceptive effect of venlafaxine with tramadol by tail-flick test in animal model of mice. *Natl. J. Physiol. Pharm. Pharmacol*. 2021;11(6):633–637. DOI: 10.5455/njppp.2021.11.11307202001022021.

17. Gregory N.S., Harris A.L., Robinson C.R., Dougherty P.M., Fuchs P.N., Sluka K.A. An overview of animal models of pain: disease models and outcome measures. *J. Pain*. 2013;14(11):1255–1269. DOI: 10.1016/j.jpain.2013.06.008.
18. Chayka A.V., Cheretaev I.V., Khusainov D.R. Methods of experimental preclinical testing of the analgesic effect of various factors in laboratory rats and mice. *Scientific Notes of V.I. Vernadsky Crimean Federal University*. 2015;1(67):161–173 (in Russ.).
19. Santos L.H., Feres C.A., Melo F.H., Coelho M.M., Nothenberg M.S., Oga S. et al. Anti-inflammatory, antinociceptive and ulcerogenic activity of a zinc-diclofenac complex in rats. *Braz. J. Med. Biol. Res.* 2004;37(8):1205–1213. DOI: 10.1590/S0100-879X2004000800011.
20. Bondarenko D.A., D›yachenko I.A., Skobtsov D.I., Murashev A.N. *In vivo* models for the study of analgesic activity. *Biomedicine*. 2011;2:84–94 (in Russ.).
21. Takeuchi K. Pathogenesis of NSAID-induced gastric damage: importance of cyclooxygenase inhibition and gastric hypermotility. *World J. Gastroenterol.* 2012;18(18):2147–2160. DOI: 10.3748/wjg.v18.i18.2147.

Authors' contribution

Aliforenko A.E., Bykov V.V., Bykova A.V., Motov V.S. – conception and design, carrying out of the experiment, analysis and interpretation of the data. Pavlovsky V.I. – chemical synthesis of the PAV-0056 compound. Khazanov V.A., Stankevich S.A. – justification of the manuscript, critical revision of the manuscript for important intellectual content. Vengerovskii A.I. – final approval of the manuscript for publication.

Authors' information

Aliforenko Anastasia E. – Post-Graduate Student, Pharmacology Division, Siberian State Medical University; Junior Researcher, IPHAR LLC, Tomsk, clin_dep2@iphar.ru, http://orcid.org/0000-0003-1656-8429

Bykov Vladimir V. – Cand. Sci. (Med.), Senior Lecturer, Pharmacology Division, Siberian State Medical University; Head of the Department of Pharmacological Research, IPHAR LLC, Tomsk, preclin5_dep@iphar.ru, http://orcid.org/0000-0002-5145-2184

Bykova Arina V. – Cand. Sci. (Biology), Senior Researcher, IPHAR LLC, Tomsk, preclin7_dep@iphar.ru, http://orcid.org/0000-0002-8495-8560

Motov Valery S. – Researcher, IPHAR LLC, Tomsk, preclin13_dep@iphar.ru, http://orcid.org/0000-0002-0197-7521

Stankevich Sergey A. – Cand. Sci. (Med.), Scientific Head of Preclinical Research, IPHAR LLC, Tomsk, project_dep@iphar.ru, http://orcid.org/0000-0003-1313-4967

Pavlovsky Victor I. — Dr. Sci. (Chemistry), Professor, Kizhner Research Center, Tomsk Polytechnic University; Leading Researcher, IPHAR LLC, Tomsk, med_chem@iphar.ru, http://orcid.org/0000-0002-4583-6245

Khazanov Veniamin A. – Dr. Sci. (Med.), Professor, Director General, IPHAR LLC, Tomsk, gen_dir@iphar.ru, http://orcid.org/0000-0002-8833-785X

Vengerovskii Alexander I. – Dr. Sci. (Med.), Professor, Pharmacology Division, Siberian State Medical University, Tomsk, pharm-sibgmu@rambler.ru, http://orcid.org/0000-0001-5094-3742

(✉) **Bykov Vladimir V.,** preclin5_dep@iphar.ru

Received 11.08.2022;
approved after peer review 05.09.2022;
accepted 08.12.2022