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Risk of developing drug abuse in administration of a new hexaazaisowurtzitane derivative-based analgesic (experimental study)

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ABSTRACT

The aim of the study was to assess the probability of developing withdrawal syndrome caused by discontinuation of 5-day administration of thiowurtzine with naloxone challenge test in the experiment.

Materials and methods. The test sample of the analgesic "Thiowurtzine, capsule 120 mg" served as the study object. The active pharmaceutical ingredient is an organic, low molecular weight compound 4-(3,4-dibromothiophene carbonyl)-2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazatetracyclo [5,5,0,$0^{3,11}$,$0^{5,9}$]dodecane that was first synthesized according to computer modeling results at the IPCET SB RAS (Biysk).

The likelihood of developing physical dependence was explored by *per os* administration of thiowurtzine and the reference drug tramadol twice a day for 5 days as follows: 1) at 9 a.m. – thiowurtzine 50 mg / kg and tramadol 10 mg / kg, at 3 p.m. – thiowurtzine 50 mg / kg and tramadol 10 mg / kg; 2) at 9 a.m. – thiowurtzine 50 mg / kg and tramadol 10 mg / kg, at 3 p.m. – thiowurtzine 75 mg / kg and tramadol 15 mg / kg; 3) at 9 a.m. – thiowurtzine 75 mg / kg and tramadol 15 mg / kg, at 3 p.m. – thiowurtzine 75 mg / kg and tramadol 15 mg / kg; 4) at 9 a.m. – thiowurtzine 100 mg / kg and tramadol 20 mg / kg, at 3 p.m. – thiowurtzine 100 mg / kg and tramadol 20 mg / kg; 5) at 9 a.m. – thiowurtzine 100 mg / kg and tramadol 20 mg / kg, at 3 p.m. – naloxone 10 mg / kg subcutaneously.

In all the groups, the intensity of the withdrawal syndrome was studied by specific features in outbred male CD1 mice. During one hour following the naloxone injection, health of mice was assessed according to dominant abstinence components and recessive traits of mild withdrawal syndrome. Two hours after the naloxone injection, the number of mice with negative body weight gain was determined. 24 hours after discontinuation of test compound administration, the open-field test was used to determine the impact on animal behavioral patterns (horizontal and vertical motor and exploratory activity, emotionality and its vegetative manifestations). The hot plate test was carried out to measure the analgesic activity (55˚). The criterion of the withdrawal syndrome severity was a decrease in the number of jumping reactions, changes in the general condition of the animals, stimulation of motor activity, manifestations of hyperalgesia, and a decrease in body weight.

Results. No dominant abstinence components and recessive signs of withdrawal syndrome were detected in animals from the thiowurtzine groups. The data obtained in the study (orientation and exploratory behavior, motor activity, emotionality and its vegetative manifestations, grooming, etc.) allow to conclude that thiowurtzine causes no physical dependence in animals after discontinuation of its 5-day administration with naloxone challenge test, as opposed to the reference drug naloxone. A positive disinhibition effect of this analgesic was revealed due to the activated orientation and exploratory behavior (stress caused by the new environment) in the conditions of the open-field test. The animals showed no manifestations of hyperalgesia in the hot plate test. The animals treated with thiowurtzine did not demonstrate any changes in the body weight.

Conclusion. The obtained results prove that thiowurtzine is a non-narcotic analgesic. It evokes no side effects typical of opioid analgesics (tramadol), including development of physical dependence and withdrawal syndrome

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following naloxone challenge test. Previous *in vivo* and *in silico* studies (docking, molecular modeling, molecular dynamics simulation) on the multi-target mechanism of thiowurtzine explain the absence of its morphine-like effect by the fact that the major targets of the analgesic are TRPA1 receptors and voltage-gated Ca^{2+} channels. With a high degree of probability, the conclusions made herein predict no drug abuse development when thiowurtzine is used in the clinical setting. Absence of ulcerotoxicity found earlier will enable to administer thiowurtzine in long-term cycles for chronic pain syndrome.

Keywords: hexaazaisowurtzitane, thiowurtzine, analgesic activity, withdrawal syndrome, physical dependence, naloxone, tramadol

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Риск развития лекарственной зависимости при применении нового анальгетика на основе производного гексаазаизовюрцитана (экспериментальное исследование)

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РЕЗЮМЕ

Целью данного исследования явилось изучение вероятности развития синдрома отмены, вызываемого прекращением 5-суточного введения тиовюрцина по схеме с провокацией налоксоном в эксперименте.

Материалы и методы. Объект исследования экспериментальный образец анальгетика «Тиовюрцин, капсула 120 мг». Активная фармацевтическая субстанция представляет собой органическое низкомолекулярное соединение 4-(3,4-дибромтиофенкарбонил)-2,6,8,12-тетраацетил-2,4,6,8,10,12гексаазатетрацикло [5,5,0,$0^{3,11}$,$0^{5,9}$]додекан, синтезированное впервые по результатам компьютерного моделирования в ИПХЭТ СО РАН **(**г. Бийск**).**

Возможность развития физической зависимости исследовали при введении тиовюрцина и референс-препарата трамадола *per os* 2 раза/сут в течение 5 сут по схеме: 1) в 9 ч – тиовюрцин 50 мг/кг и трамадол 10 мг/кг, в 15 ч – тиовюрцин 50 мг/кг и трамадол 10 мг/кг соответственно; 2) в 9 ч – тиовюрцин 50 мг/кг и трамадол 10 мг/кг, в 15 ч – тиовюрцин 75 мг/кг и трамадол 15 мг/кг; 3) в 9 ч – тиовюрцин 75 мг/кг и трамадол 15 мг/кг, в 15 – тиовюрцин 75 мг/кг и трамадол 15 мг/кг, 4) в 9 ч – тиовюрцин 100 мг/кг и трамадол 20 мг/кг,

в 15 ч – тиовюрцин 100 мг/кг и трамадол 20 мг/кг; 5) в 9 ч – тиовюрцин 100 мг/кг и трамадол 20 мг/кг, в 15 ч – налоксон в дозе 10 мг/кг подкожно.

Во всех группах интенсивность синдрома отмены изучали по специфическим признакам у аутбредных мышей-самцов сток CD1. На протяжении 1-го ч после введения налоксона регистрировали основные компоненты абстиненции и рецессивные признаки легкого течения синдрома отмены. Через 2 ч после инъекции налоксона определяли число мышей с отрицательным привесом массы тела. Через 24 ч после отмены тестируемых веществ исследовали степень воздействия на структуру поведения животных (горизонтально-вертикальную двигательную и исследовательскую активности, эмоциональность и ее вегетативные проявления) в тесте «открытое поле», наличие анальгетического действия в тесте «Горячая пластина» (55˚). Критерием выраженности синдрома отмены считали уменьшение числа прыжковых реакций, изменение общего состояния животных, стимулирование двигательной активности, проявление гипералъгезии, снижение массы тела у мышей.

Результаты. «Доминантных» компонентов абстиненции и рецессивных признаков синдрома отмены у животных из групп введения тиовюрцина не зафиксировано. Совокупность полученных данных (ориентировочно-исследовательское поведение, двигательная активность, эмоциональность по ее вегетативным проявлениям, груминг и т.д.) позволяет заключить, что тиовюрцин после отмены 5-суточного введения по схеме с провокацией налоксоном не вызывает развития физической зависимости в отличие от референс-препарата трамадола. Выявлено позитивное растормаживающее действие анальгетика за счет активации ориентировочно-исследовательского компонента поведения (стресс новизны) в условиях теста «открытое поле». Отсутствовало проявление гипералъгезии у животных в тесте «горячая пластина». Изменения массы тела животных, получавших тиовюрцин, не наблюдалось.

Заключение. Представленные результаты свидетельствуют о том, что тиовюрцин не является наркотическим анальгетиком. Он не проявляет побочных эффектов, типичных для обезболивающих средств с опиоидным компонентом механизма действия (трамадол), прежде всего развития физической зависимости и формирования синдрома отмены. В подтверждение вышесказанного проведенные ранее исследования *in vivo* и *in silico* (докинг, молекулярное моделирование, моделирование молекулярной динамики) мультитаргетного механизма действия тиовюрцина объясняют отсутствие его морфиноподобного действия тем, что основными мишенями анальгетика являются TRPA1-рецепторы и потенциал-зависимые Ca^{2+}ионные каналы. Полученные выводы позволяют с высокой степенью вероятности прогнозировать отсутствие лекарственной зависимости при клиническом применении тиовюрцина, а выявленное ранее отсутствие гастротоксичности предполагает возможность использования анальгетика продолжительными курсами при хроническом болевом синдроме.

Ключевые слова: гексаазаизовюрцитан, тиовюрцин, анальгетическая активность, синдром отмены, физическая зависимость, налоксон, трамадол

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

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INTRODUCTION

Many acute and chronic diseases, injuriesб and medical interventions are associated with pain, which dramatically impairs the quality of life and requires the use of painkillers. According to experts from the International Association for the Study of Pain, about 20% of humans suffer from chronic pain syndrome due to low efficacy of symptomatic therapy. According to statistics, the number of

cancer patients, patients diagnosed with myocardial infarction and coronary artery disease, as well as patients with various injuries reaches up to 13 million people a year in the Russian Federation. In this respect, analgesics for treatment of severe and excruciating pain of various etiology (including chronic pain) and opioid antagonists are strategically important categories of medicines [1–3]. At the same time, according to the estimates of anesthesiologists and expert analysts from the Russian pharmaceutical market, only about 10% of the demand for next-generation analgesics (enhanced opioids, combined analgesics, etc.) in the clinical setting is addressed. Moreover, the existing problem of side effects (gastro-, nephro-, hepato-, cardio-, and hematotoxicity, teratogenicity, mutagenicity, development of physical and psychological addiction) is still of great medical and social importance due to high availability and widespread use of painkillers of different groups [1–3]. All the above factors determine the need for search, development, and practical implementation of conceptually new analgesics with low toxicity acting on the molecular mechanisms of pain generation.

Preclinical studies of an innovative analgesic (hereinafter referred to as thiowurtzine, TWZ) based on the 2,4,6,8,10,12hexaazatetracyclo[5,5,0,$0^{3,11}$,$0^{5,9}$] dodecane (hexaazaizowurzitane) derivative for treatment of pain syndrome of different etiology are being currently carried out at Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk NIMC. It has been found that the test sample "Thiowurtzine, capsule 120 mg" has low toxicity and LD_{50} 150–5000 mg / kg (hazard class III in line with GOST R 12.1.007-76). Its pronounced analgesic effect was demonstrated in the hot-plate test of nociception, acute visceral and deep somatic pain model (acetic acid-induced writhing test), chemogenic pain model (formalin test), TRPV1 receptor sensitivity model (capsaicin test), and Randall – Selitto paw pressure test [4, 5]. The use of specific pharmacological analyzers allowed us to hypothesize the involvement of the kappa – opioid system and TRP receptors in the antinociceptive effect of thiowurtzine with a probable impact on serotonin and GABAergic structures of the central nervous system and T-type calcium (Ca^{2+}) channels [6, 7]. The discovered multi-target analgesic mechanism of thiowurtzine is manifested at different levels of perception, implementation, and modulation of nociceptive activity, which requires research on the probability of drug abuse development due to drug administration.

The present study is aimed at exploring the likelihood of withdrawal syndrome development due to discontinuation of the 5-day administration of thiowurtzine with naloxone challenge test in the experiment.

MATERIALS AND METHODS

The experiments were conducted on 60 outbred male CD1 mice (weight 20.5 g, age 7–8 weeks). The animals were obtained from the Department of Experimental Biomodeling at Goldberg Research Institute of Tomsk NRMC (Certificate of Animal Health). Animal maintenance and experiment design were approved by the Bioethics Committee at Goldberg Research Institute (JACUC protocol No. 96092015 of 16.09.2015) and complied with the Directive 2010/63/EU of the European Parliament and the Council of the European Union on the Protection of Animals Used for Scientific Purposes and GOST No. 33044-2014 "Principles of Good Laboratory Practice" of 01.08.2015.

The animals were randomly classified into groups using body weight (± 10%) as a criterion for classification. The mice were euthanized by cervical dislocation. The test sample "Thiowurtzine, capsule 120 mg" was the object of the study. The active pharmaceutical ingredient was an organic low molecular weight compound 4-(3,4-dibromothiophene carbonyl)-2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazatetracyclo[5,5,0,$0^{3,11}$,$0^{5,9}$]dodecane that was first synthesized using computer modeling results at IPCET SB RAS (Biysk) [5]. The previous study on the analgesic effect of thiowurtzine determined the effective therapeutic dose of the compound – 100 mg / kg intragastrically [5, 7].

To assess the withdrawal syndrome, thiowurtzine and the reference drug tramadol hydrochloride (Joint Stock Company Organica, Russian Federation) were administered *per os* twice a day (in the morning and in the evening) for 5 days as follows: 1) at 9 a.m. – thiowurtzine 50 mg / kg and tramadol 10 mg / kg, at 3 p.m. – thiowurtzine 50 mg / kg and tramadol 10 mg / kg; 2) at 9 a.m. – thiowurtzine 50 mg / kg and tramadol 10 mg / kg, at 3 p.m. – thiowurtzine 75 mg / kg and tramadol 15 mg / kg; 3) at 9 a.m. – thiowurtzine 75 mg / kg and tramadol 15 mg / kg, at 3 p.m. – thiowurtzine 75 mg / kg and tramadol 15 mg / kg; 4) at 9 a.m. – thiowurtzine 100 mg / kg and tramadol

20 mg / kg, at 3 p.m. – thiowurtzine 100 mg / kg and tramadol 20 mg / kg; 5) at 9 a.m. – thiowurtzine 100 mg / kg and tramadol 20 mg / kg, at 3 p.m. – naloxone 10 mg / kg subcutaneously [8–10].

Naloxone (Moscow Pharmaceutical Factory, Russian Federation) is a non-selective opioid antagonist. The animals were divided into 6 groups for the experiments. Group 1: control (purified water) according to the regimen. Group 2: control (purified water) according to the regimen + naloxone. Group 3: thiowurtzine (decapsulated) according to the regimen. Group 4: thiowurtzine (decapsulated) according to the regimen + naloxone. Group 5: tramadol according to the regimen. Group 6: tramadol according to the regimen + naloxone.

During 1 hour following the naloxone injection, the overall health status of mice was assessed according to dominant withdrawal syndrome components and recessive traits of mild withdrawal syndrome (abnormal acoustic and corneal reflexes, the presence of convulsions, writhing movements, tremor as if beating a drum, ptosis, jumping, shaking, chattering teeth, Straub reaction, itching, sneezing, lateral position). The weight of the rodents was measured during the randomization and 2 h after the naloxone injection. 24 hours after withdrawal of the test compounds, the open-field test was used to determine the degree of impact on animal behavioral patterns (emotionality, horizontal and vertical motor and exploratory activity) [8, 11]. The mice were placed at the center of the open-field arena. Then the behavioral patterns of each animal were recorded for 2 min: horizontal activity (the number of crossed squares); vertical activity (the number of rearings with and without support of the cage edge); exploratory activity (the number of holepoking movements); emotional reaction and its vegetative manifestations (defecation); grooming.

Inactive animals with more intense defecation in the open-field maze were considered more emotional than animals who moved a lot but showed less intense defecation. Statistically significant variation in the parameters of horizontal and vertical motor activity was considered a criterion of sedative or stimulating effect. The analgesic activity was studied using a Hot Plate Analgesia Meter (Columbus Instruments, USA) at 55ºC. Latency of the pain response was recorded via animal's licking their hind paws, which is a sign that the pain threshold was reached. The analgesic effect was presented as the average latency in the group, while a significant increase in the response latency after the administration of the compounds was considered a hyperalgesia criterion [8].

The statistical analysis of the obtained data was performed using *Statistica 6.0*. The mean (X) and standard deviation (m) were calculated for all data. Along with the value of n (number of variants), they are presented in the final tables. The intergroup differences were checked using the non-parametric Kruskal – Wallis and Mann – Whitney – Wilcoxon (U) tests. The Fisher's angular transformation criterion (φ) was used to compare frequencies. The differences were considered statistically significant at $p \leq 0.05$ [8].

RESULTS

The control experiments demonstrated no signs of physical dependence while the withdrawal syndrome with naloxone challenge test was examined (table 1).

Table 1

Manifestation of reflexes in outbred male CD1 mice after discontinued administration of thiowurtzine (50–100 mg / kg) and tramadol (10–20 mg / kg) with naloxone challenge test (10 mg / kg s/c)

Reflex	Control, $n = 10$	Control + naloxone, $n = 10$	Thiowurtzine, $n = 10$	Thiowurtzine + naloxone, $n = 10$	Tramadol, $n = 10$	Tramadol + naloxone, $n = 10$
	Number of animals with reflex manifestations, %					
Acoustic	100	100	100	100	100	100
Corneal	100	100	100	100	100	100
Convulsions	0	0	0	0	30*	20*
Tremor	0	0	0	0	20*	20*
Ptosis	0	0	0	0	0	10
Jumping	0	0	0	0	0	10
Shaking	0	0	0	0	20*	0
Chattering teeth	0	0	0	0	0	0

Table 1 (continued)

Reflex	Control, $n = 10$	Control + naloxone, $n = 10$	Thiowurtzine, $n = 10$	Thiowurtzine + naloxone, $n = 10$	Tramadol, $n = 10$	Tramadol + naloxone, $n = 10$
	Number of animals with reflex manifestations, %					
Straub reaction	0	0	0	0	0	20*
Itching	100	100	100	100	100	100
Sneezing	0	0	0	0	0	0
Lateral position	0	0	0	0	0	0

*$p < 0.01$ compared with the control (Fisher's test)

Recessive signs of abuse were recorded in the animals treated with tramadol: tremor (20%), shaking (20%), and convulsions as the withdrawal syndrome component (30%) (Table 1). During tramadol administration according to the regimen, naloxone evoked not only such signs of mild withdrawal syndrome as shaking (20%), tremor as if beating a drum (20%), and ptosis (10%), but also jumping activity (10%), Straub reaction (20%), and convulsions (20%), which are dominant withdrawal syndrome components (Table 1). The mice treated with thiowurtzine according to the regimen in combination with naloxone had none of the physical dependence signs.

The study results for exploratory activity of the animals in the open-field test suggest that thiowurtzine after administration according to the regimen with naloxone challenge test did not change the number of vertical postures and the number of holepoking movements throughout the entire observation period (Table 2).

Table 2

Effect of cycle administration of thiowurtzine according to the ascending dose regimen (50–100 mg / kg) on behavioral patterns of outbred male CD1 mice in the open-field test against the background of administration discontinuation with naloxone challenge test (10 mg / kg s / c), $X \pm m$

Study group (number of animals, $n = 10$)	Overall motor activity	Horizontal activity	Vertical activity	Hole exploratory behavior	Grooming	Defecation	Asymmetry coefficient
1st min of observation							
1.Water control (according to the regimen)	42.3 ± 4.0	25.5 ± 2.9	3.1 ± 1.1	13.5 ± 1.9	0.2 ± 0.1	0 ± 0	60.6 ± 3.9
2. Control (according to the regimen) + naloxone	43.1 ± 3.8	28.3 ± 2.8	3.4 ± 0.8	10.7 ± 1.8	0.1 ± 0.1	0.6 ± 0.3	65.4 ± 3.1
3. Thiowurtzine (according to the regimen)	44.0 ± 4,2	33.4 ± 0.1	2.9 ± 0.7	10.9 ± 1.8	0 ± 0	0.2 ± 0.2	66.8 ± 4.1
4. Thiowurtzine (according to the regimen) + naloxone	54.1 ± 5.1*	35.0 ± 4.0*	4.0 ± 1.3	15.0 ± 1.5	0 ± 0	0.1 ± 0.1	63.5 ± 2.3
5. Tramadol (according to the regimen)	53.6 ± 4.8	37.0 ± 3.6*	4.6 ± 1.1	11.6 ± 1.7	0 ± 0	0.4 ± 0.3	69.3 ± 3.4
6. Tramadol (according to the regimen) + naloxone	38.2 ± 3.3+	25.3 ± 3.0+	1.9 ± 0.8+	10.5 ± 1.7	0.3 ± 0.2	0.2 ± 0.1	65.8 ± 5.3
2nd–3rd min of observation							
1. Water control (according to the regimen)	73.3 ± 7.1	35.3 ± 4.1	6.5 ± 2.0	30.8 ± 4.1	0.6 ± 0.3	0.1 ± 0.1	48.6 ± 3.4
2. Control (according to the regimen) + naloxone	79.9 ± 5.6	40.1 ± 3.0	8.1 ± 1.6	30.3 ± 3.4	0.9 ± 0.3	0.5 ± 0.3	51.1 ± 3.6
3. Thiowurtzine (according to the regimen)	78.9 ± 9.0	45.7 ± 5.7	6.1 ± 1.0	26.0 ± 3.4	0.9 ± 0.2	0.2 ± 0.1	57.9 ± 3.3
4. Thiowurtzine (according to the regimen) + naloxone	96.9 ± 9.2*	52.3 ± 6.12*	6.5 ± 1.2	37.2 ± 3.7	0.5 ± 0.2	0.4 ± 0.4	53.8 ± 3.6
5. Tramadol (according to the regimen)	102.0 ± 12.1	53.9 ± 9.1	5.9 ± 1.2	41.5 ± 6.1	0.6 ± 0.2	0.4 ± 0.3	51.6 ± 4.2
6. Tramadol (according to the regimen) + Naloxone	83.5 ± 9.45	48.7 ± 7.8	3.8 ± 1.0*#	30.2 ± 2.5	0.4 ± 0.2	0.4 ± 0.2	49.5 ± 6.3
Overall outcome, 1st–3rd min of observation							
1. Water control (according to the regimen)	115.6 ± 10.3	60.8 ± 6.5	9.6 ± 2.9	44.3 ± 5.4	0.8 ± 0.3	0.1 ± 0.1	52.6 ± 2.7
2. Control (according to the regimen) + naloxone	123.0 ± 9.2	68.4 ± 5.1	11.5 ± 2.2	41.0 ± 4.7	1.0 ± 0.3	1.1 ± 0.6	56.1 ± 2.6
3. Thiowurtzine (according to the regimen)	122.9 ± 12.2	75.7 ± 8.3	9.0 ± 1.5	36.9 ± 5.0	0.9 ± 0.2	0.4 ± 0.3	61.1 ± 3.1
4. Thiowurtzine (according to the regimen) + naloxone	151.0 ± 12.8	87.3 ± 8.1*	10.5 ± 2.2	52.2 ± 4.2	0.5 ± 0.2	0.5 ± 0.5	58.0 ± 2.1
5. Tramadol (according to the regimen)	155.9 ± 15.6*	90.9 ± 11.7*	10.5 ± 2.1	53.1 ± 6.7	0.6 ± 0.2	0.8 ± 0.4	57.4 ± 2.5
6. Tramadol (according to the regimen) + naloxone	122.0 ± 12.2+	74.0 ± 10.6	5.7 ± 1.4#+	40.7 ± 3.7	0.7 ± 0.3	0.6 ± 0.3	66.9 ± 8.7

*$p < 0.05$ in comparison with water control, #$p < 0.05$ in comparison with the Control + naloxone group, +$p < 0.05$ in comparison with the Tramadol group (Mann – Whitney – Wilcoxon test).

In addition, the absence of statistically significant changes in the grooming and defecation parameters indicates that the drug did not affect the emotional level of the animals. A 1.3-fold increase in the overall motor activity ($p < 0.05$) due to a 1.4-fold increase in the horizontal motor activity (by the number of squares crossed) ($p < 0.05$) during the 1st min of observation and a 1.3-fold ($p < 0.05$) and 1.5-fold increase ($p < 0.05$) in the 2nd–3rd min of the experiment, respectively, relative to similar control data is worth noting.

The revealed moderate stimulating effect of thiowurtzine on the horizontal activity can be explained by the activated orientation and exploratory behavior of mice due to the disinhibition effect of the drug on behavioral reactions of the animals under stress caused by new conditions [8, 11]. The data obtained for all the analyzed parameters (horizontal, vertical and exploratory activity, emotionality by its vegetative manifestations, grooming, etc.) allow us to state that withdrawal syndrome was not formed. In contrast, a statistically significant decrease in overall, horizontal, and vertical motor activity of the mice treated with tramadol with naloxone challenge test, in comparison with similar parameters in the animals of the tramadol group, complied with the sedative activity criterion and indicated withdrawal syndrome development (Table 2).

The data presented in Table 3 allow to conclude that the animals that received naloxone injections after tramadol administration was discontinued developed hyperalgesia, since the response latency exceeded that of the Tramadol group by 1.2 times ($p < 0.05$).

The baseline value for thiowurtzine-suppressed pain sensitivity in the case of a single injection was 50% in the hot plate test, while 24 h after 5-day administration of thiowurtzine was discontinued, the antinociceptive effect of the drug appeared to be statistically significant and reached 23.9% relative to the water control (Table 3). In the same period of observation, the naloxone challenge test resulted in a 21% decrease in the thiowurtzine activity when compared to the same effect in the above-mentioned group, which allows for the conclusion that the animals did not develop hyperalgesia.

The body mass of test animals is one of the important parameters for veterinary monitoring in a long-term experiment.

Table 3

Body mass index of outbred male CD1 mice and antinociceptive response value in the hot plate test in withdrawal syndrome with naloxone challenge test, $X \pm m$

Animal group (number of animals, $n = 10$)	Body mass of mice, g		Response latency development, sec
	before injections	after injections were discontinued	
1. Control, purified water (according to the regimen)	25.5 ± 0.7	25.3 ± 0.9	17.6 ± 2.2
2. Control, purified water (according to the regimen) + naloxone, 10	25.6 ± 0.5	25.7 ± 0.4	17.9 ± 2.0
3. Thiowurtzine (according to the regimen)	25.5 ± 0.6	26.2 ± 0.4	21.8 ± 2.7*
4. Thiowurtzine (according to the regimen) + naloxone, 10	25.6 ± 0.5	26.2 ± 0.5	17.2 ± 1.4
5. Tramadol (according to the regimen)	25.6 ± 0.5	25.5 ± 0.6	17.8 ± 1.3

* $p < 0.05$ in comparison with the Thiowurtzine + naloxone group; $^{\#}p < 0.05$ in comparison with the Control + naloxone group; $^{+}p < 0.05$ in comparison with the Tramadol group (Mann – Whitney – Wilcoxon test).

It can indicate an overall adverse effect of a test compound on metabolism, as well as undesirable and side effects when combined with toxicity testing data. No negative changes in the body mass of the test animals treated with thiowurtzine with naloxone challenge test, which induces withdrawal syndrome, were noted (Table 3).

CONCLUSION

The data obtained on the degree of impact on the animal behavior in the open-field test (horizontal activity, vertical and exploratory activity, emotionality and its vegetative manifestations, grooming, etc.) allow for the conclusion that thiowurtzine causes no sedation or euphoria in animals after discontinuation of its 5-day administration regimen with naloxone challenge test. A positive feature of this analgesic is its disinhibition effect due to the activated orientation and exploratory behavior in the afferentation conditions of the open-field test. It should be noted that dominant withdrawal syndrome components and recessive signs of withdrawal syndrome were not detected in the animals after the thiowurtzine administration, as opposed to the tramadol-dependent mice. The mice that received the analgesic did not show a decrease in body mass, which points to the absence of a toxic effect on the metabolism in the test animals. Discontinuation of the thiowurtzine administration with naxolone challenge test caused no hyperalgesia in the animals in the hot plate test.

The findings presented herein indicate that thiowurtzine evokes no side effects typical of opioid analgesics (tramadol), including physical dependence and withdrawal syndrome development following naloxone challenge test. Previous *in vivo* and *in silico* studies (docking, molecular modeling, molecular dynamics simulation) on the multi-target mechanism of thiowurtzine explain the absence of its morphine-like effect by the fact that the major targets of the analgesic are TRPA1 receptors and voltage-gated Ca^{2+} channels [6, 12]. With a high degree of probability, the conclusions made herein predict no drug abuse development when thiowurtzine is used in the clinical setting. The absence of ulcerotoxicity found earlier will enable to administer thiowurtzine in long-term cycles with no risk of gastrotoxicity for patients with chronic pain syndrome.

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Authors contribution

Krylova S.G. – conception and design of the experiments, participation in the experiments, critical revision of the manuscript for important intellectual content. Lopatina K.A. – carrying out of the experiments, statistical analysis, writing of the article. Zueva E.P. – critical revision of the manuscript for important intellectual content. Povetieva T.N. – conception and design of the experiments, participation in the experiments. Suslov N.I. – critical revision of the manuscript for important intellectual content. Nesterova Y.V. – participation in the experiments, statistical analysis. Afanasieva O.G., Kiseleva E.A., Kulpin P.V. – participation in the experiments. Sysolyatin S.V. – organization of the research object synthesis. Kulagina D.A. – provision of the research object and its synthesis. Zhdanov V.V. – analysis of the results of computer activity prediction, final approval of the manuscript for publication.

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