RESEARCH ARTICLE
ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

STUDY OF RAPITALAM INFLUENCE ON OXOTREMORINE-INDUCED TREMOR

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Received: 04.12.18 Accepted for publication: 03.03.2019

Parkinson's disease is the second most common (after Alzheimer's) neurodegenerative disease. All over the world, there is a search for new drugs aimed at the treatment of Parkinson's disease. Till up to the present, there is no "ideal" medicine that can completely cure this disease and has minimal adverse side effects. Belgorod research institute of pharmacology of living systems is studying Rapitalam, a new drug for the treatment of tremulous Parkinson's disease. This is an agonist of the mGluR4 group of metabotropic receptors.

***The aim** of the article is to study Rapitalam influence on the oxotremorine-induced tremor in rats.*

***Methods.** The study comprised 60 rats (6 groups of 10 males), which were administered intragastrically with the studied substances for 10 days. All the animal groups except Control group 1, were administered with Rapitalam and the reference drug Levodopa. 30 minutes after Rapitalam and Levodopa, they were administered abdominally with the solution of Oxotremorine at the dose of 1.5 mg/kg. The animals of Control group 1, instead of Oxotremorine, were similarly administered with a solvent of 0.9% sodium chloride in the equivalent volume.*

***Results.** In comparison with the reference group, Rapitalam at the dose of 3 mg/kg significantly reduced the severity of tremor 50 min. after its administration. The same effect took place 30 min after the administration of Oxotremorine at the dose of 10 mg/kg. At the dose of 3 and 10 mg/kg, Rapitalam also decreased the number of rats in the group (in %) with the signs of tremor 60 min. and 50 min. after the administration of Oxotremorine, respectively.*

***Conclusion.** The study revealed that Rapitalam has a pronounced anti-tremor effect. Its administration at the studied doses reduced the symptoms of Oxotremorine-induced tremor in rats.*

***Keywords**: Parkinson's Disease; mGluR4; Rapitalam; Oxotremorine-induced tremor; Levodopa*

ИЗУЧЕНИЕ ВЛИЯНИЯ РАПИТАЛАМА НА ОКСОТРЕМОРИН-ИНДУЦИРОВАННЫЙ ТРЕМОР

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Поступила в редакцию: 04.12.18 Принята к печати: 03.03.2019

***Аннотация.** Во всем мире идет поиск новых препаратов для лечения болезни Паркинсона, которая является вторым по распространенности нейродегенеративным заболеванием после болезни Альцгеймера. До настоящего времени «идеального» лекарственного средства, обладающего минимальными побочными эффектами и способного полностью вылечить пациентов с данным заболеванием, не существует. В научно-исследовательском институте «Фармакология живых систем» (г. Белгород) изучается новое лекарственное средство для лечения дрожательной формы болезни Паркинсона – Рапиталам, механизм действия которого заключается в активации метаботропных рецепторов группы mGluR4.*

***Цель** – изучить влияние Рапиталама на оксотреморин-индуцированный тремор у крыс.*

For citation: N.V. Avdeeva. Study of rapitalam influence on oxotremorine-induced tremor. *Pharmacy & Pharmacology*. 2019;7(2): 84-89. **DOI:** 10.19163/2307-9266-2019-7-2-84-89

Для цитирования: Н.В. Авдеева. Изучение влияния рапиталама на оксотреморин-индуцированный тремор. *Фармация и фармакология*. 2019;7(2): 84-89. **DOI:** 10.19163/2307-9266-2019-7-2-84-89

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DOI: 10.19163/2307-9266-2019-7-2-84-89

Методы. *В исследование было включено 60 крыс мужского пола, которые были разделены на 6 групп по 10 особей. Животным внутрижелудочно в течение 10 дней вводили исследуемые соединения. Всем группам животных, кроме группы Контроль 1, через 30 мин после введения Рапиталама и препарата сравнения Леводопа внутрибрюшинно вводили раствор оксотреморина в дозе 1,5 мг/кг. Животным из группы Контроль 1 аналогичным образом вместо оксотреморина вводили растворитель (0,9% раствор натрия хлорида) эквивалентном объеме.*
Результаты. Введение крысам исследуемого соединения Рапиталам в дозе 3 мг/кг вызывало достоверное в сравнении с группой контроля снижение выраженности тремора через 50 мин, в дозе 10 мг/кг уже через 30 мин после введения оксотреморина. Также введение Рапиталама в дозе 3 и 10 мг/кг приводило к уменьшению количества крыс (выраженное в %) в группе с проявлениями тремора через 60 мин и 50 мин соответственно.
Заключение. *В результате исследования выявлено, что Рапиталам обладает выраженным антитреморным эффектом, что подтверждалось снижением проявлений оксотреморин-индуцированного тремора у крыс после введения исследуемого препарата.*
Ключевые слова: *болезнь Паркинсона, mGluR4, Рапиталам, оксотреморин-индуцированный тремор, Леводопа*

INTRODUCTION

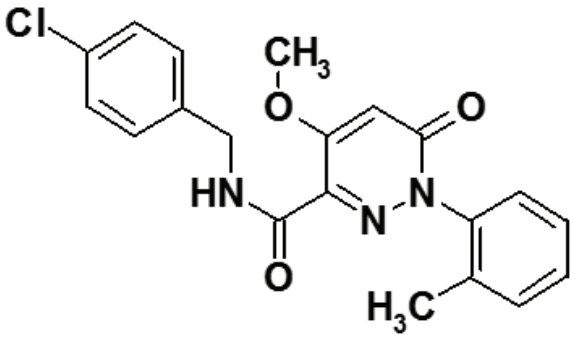
Parkinson's disease is a chronic disease characterized by dysfunction of the nervous system and movement disorders. The disease is progressive, that's why it is necessary to diagnose it as soon as possible and choose the necessary therapy. Drugs of different groups are selected individually in accordance with clinical manifestations of the disease. However, when prescribing the drugs, the problem of drug habituation and, consequently, necessity for dose escalation exists [1]. For this reason, at the early stages, doctors prescribe more sparing drugs in minimal doses. Unfortunately, there are none of the drugs for the treatment of Parkinson's disease which can completely cure this pathology. That is why the discovery of new therapeutic targets and drugs that can slow down the progression of neurodegeneration, remains an urgent task [2].

Metabotropic receptors (mGluRs) can have a neuromodulatory effect on both glutamatergic and GABA-ergic neurotransmission. That is very interesting for the development of new mGluR ligands which can be used to treatment of various neurological and mental disorders [3-5]. Metabotropic receptors mGLuR4 have been paid a lot of attention as a therapeutic target [6-8]. According to the literature data, a great number of mGLuR4 are presented in the synapses of striapallidal paths, in particular, in the neurons of the internal Globus pallidus segment and pars reticulate of the Substantia nigra [9].

Activation of these receptors leads to the reinforcement of GABA-ergic inhibition of the thalamus glutamatergic neurons by the striapallidal system, and consequently, to the correction of the imbalance between inhibitory and excitatory effects, shifted towards excitation, which is the basis of the pathogenesis of the tremulous form of Parkinson's disease [10, 11]. Previously, the mode of action of a new pharmacological substance – Rapitalam, was studied in Belgorod Research institute of pharmacology of living systems

The result of this study revealed that Rapitalam is an agonist of mGluR4 [2, 12].

THE AIM of the article is to study Rapitalam influence on the Oxotremorine-induced tremor in rats.



Structure of Rapitalam.

MATERIALS AND METHODS

Animals

The study was performed on male rats of Sprague Dawley line at 12-14 weeks of age, weighing 230-260 grams, obtained from the nursery of laboratory animals of "The Branch of the Institute of Bioorganic chemistry n.a. M.M. Shemyakin and Y.A. Ovchinnikov", Pushchino. All the animals were divided into 6 groups of 10 individuals (Table 1). The groups were formed at random using body weight as the leading feature so that the individual weight value hadn't deviated from the average value by more than 20%. Keeping the animals complied with all the rules of laboratory practice during preclinical studies in Russia. The animals were kept in the standard conditions corresponding to sanitary rules of SP 2.2.1.3218-14 "Sanitary and epidemiological requirements to the device, the equipment and the maintenance of experimental biological clinics (vivariums)" dated 29.08.2014 No 51. and GOST 33215-2014. The contents and all animal manipulations complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for experiments and other scientific purposes (Strasbourg, 1986).

Study Design

The test compound Rapitalam and the reference drug Levodopa, were administered to the animals intragastrically (per os) once a day for 10 days. The body weight of the animals was recorded just before each administration of the test and reference drugs.

Table 1 – Animal groups

Group No	Administrated drug	Number of rats
1	Control 1 (0.5% tween-80 per os, 0.9% sodium chloride intraperitoneally)	10
	Oxotremorine-induced tremor	
2	Control 2 (0.5% tween-80 per os, oxotremorine intraperitoneally)	10
3	Levodopa 50 mg/kg	10
4	Rapitalam, 1 mg/kg	10
5	Rapitalam, 3 mg/kg	10
6	Rapitalam, 10 mg/kg	10

Note: PO – intragastrically, IP – abdominally

Model of Oxotremorine-induced tremor

All the animal groups except Control group 1, were injected intraperitoneally with an Oxotremorine solution at the dose of 1.5 mg/kg 30 min after the administration of the test compound and Levodopa. The animals of Control group 1 were similarly administered with 0.9% sodium chloride in the equivalent volume. The severity of Oxotremorine-induced tremor (in points) and the time of the symptoms reduction in rats were recorded.

Methods of statistical data analysis

The intergroup *statistical* comparison was performed using the Kruskal-Wallis test with post-hocDunn test. For comparison of repeated measurements (Oxotremorin-induced tremor during the observation period), Repeatedmeasures ANOVA was used, in case of differences between the groups, the Bonferroni correction was used. The differences were determined at 0.05 significance value (GraphPad Prism 5.0).

RESULTS

The onset of the tremor was observed in the animals of all the groups, which had been administered with Oxotremorine (Table 2).

Table 2 – Latent period and tremor duration in groups of rats, M±m

	Latent period, min.	The tremor duration, min.
Control 1	0±0	0±0
Control 2	10±0*	68.8±5.8*
Levodopa 50 mg/kg	10±0*	62.2±4*
Rapitalam 1 mg/kg	10±0*	67.8±5.2*
Rapitalam 3 mg/kg	10±0*	57.8±2.8*
Rapitalam 10 mg/kg	8.9±1.1*	52.2±3.6*#

*Note: *p<0.05 in comparison with Control group 1, #p<0.05 in comparison with Control group 2 (non-parametric Kruskal-Wallis test, post-hocDunn test).*

Oxotremorine caused the increasing severity of tremor from 10-th to 20-th minutes after intra-abdominal injection to rats of Control group 2. Forty minutes after the administration of Oxotremorine the severity of tremor in the control rats began declining, and the tremor signs were not recorded beginning with 80-th min (Table 3).

In Group 3 administered with Levodopa at the dose of 50 mg/kg, there was a significant decrease in the severity of tremor 20, 30 and 50 minutes after Oxotremorine administration in comparison with Control group 2 (Table 3). A significant decrease in the % of rats with tremor in this group was registered 60 minutes after Oxotremorine administration (Table 4).

As Table 3 shows, the administration of Rapitalam at the dose of 1 mg/kg had no effect on the performance of tremor in rats. The Rapitalam doses of 3 and 10 mg/kg significantly decreased the severity of tremor. In comparison with the control group, the severity of tremor significantly decreased to 50-th min after the administration of Oxotremorine, at the dose of 3 mg/kg, and after the administration of Oxotremorine at the dose of 10 mg/kg, it significantly decreased beginning with 30-th min. Table 4 shows that Rapitalam at the doses of 3 and 10 mg/kg decreased the percentage of rats with tremor, 50 and 60 min. after the administration of Oxotremorine, respectively.

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DOI: 10.19163/2307-9266-2019-7-2-84-89

Table 3 – Points of severity of Oxotremorine-induced tremor in rats, M±m

Group	Time after Oxotremorine administration, min.											
	10	20	30	40	50	60	70	80	90	100	110	120
Control 1	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Control 2	1.8±0.3*	2±0.3*	1.9±0.4*	1.5±0.3*	1.3±0.3*	0.6±0.3*	0.4±0.2*	0.1±0.1*	0±0	0±0	0±0	0±0
Levodopa 50 mg/kg	1.4±0.2*	1.2±0.3*#	1±0.2*#	1.1±0.1*	0.6±0.2*#	0.1±0.1*	0.1±0.1*	0.1±0.1*	0±0	0±0	0±0	0±0
Rapitalam 1 mg/kg	1.9±0.3*	1.9±0.2*	1.4±0.2*	1.3±0.2*	0.9±0.1*	0.6±0.2*	0.2±0.1*	0.2±0.1*	0±0	0±0	0±0	0±0
Rapitalam 3 mg/kg	1.8±0.2*	1.8±0.2*	1.4±0.3*	1.2±0.1*	0.6±0.2*#	0.2±0.1*	0±0*	0±0*	0±0	0±0	0±0	0±0
Rapitalam 10 mg/kg	1.3±0.3*	1.4±0.2*	1±0.2*	0.8±0.1*	0.2±0.1*#	0.2±0.1*	0±0*#	0±0*	0±0	0±0	0±0	0±0

*Note: *p<0.05 in comparison with Control group 1, #p<0.05 in comparison with Control group 2 (Repeated measures ANOVA, Bonferroni correction).*

Table 4. – Percentage of severity of Oxotremorine-induced tremor in rats, M±m

Group	Time after Oxotremorine administration, min											
	10	20	30	40	50	60	70	80	90	100	110	120
Control 1	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Control 2	100±0*	100±0*	88.9±11.1*	87.5±12.5*	87.5±12.5*	50±18.9*	37.5±18.3*	12.5±12.5*	0±0	0±0	0±0	0±0
Levodopa 50 mg/kg	100±0*	100±0*	100±0*	100±0*	55.6±17.6*	11.1±11.1*#	11.1±11.1*	11.1±11.1*	0±0	0±0	0±0	0±0
Rapitalam 1 mg/kg	100±0*	100±0*	100±0*	88.9±11.1*	88.9±11.1*&	55.6±17.6*&	22.2±14.7*	22.2±14.7*	0±0	0±0	0±0	0±0
Rapitalam 3 mg/kg	100±0*	100±0*	88.9±11.1*	100±0*	55.6±17.6*	22.2±14.7*	0±0#	0±0	0±0	0±0	0±0	0±0
Rapitalam 10 mg/kg	88.9±11.1*	100±0*	77.8±14.7*	77.8±14.7*	22.2±14.7*#&	22.2±14.7*	0±0#	0±0	0±0	0±0	0±0	0±0

*Note: *p<0.05 in comparison with Control group 1, #p<0.05 in comparison with Control group 2, &p<0.05 in comparison with L-DOPA 50 mg/kg (Repeatedmeasures ANOVA, Bonferroni correction)*

Thus, the effectiveness of Rapitalam with intragastric administration for 10 days (once a day) at the doses of 3 and 10 mg/kg was shown in the model of Oxotremorine-induced tremor in rats. The test compound at the dose of 3 mg/kg caused a significant decrease in the severity of the tremor from since 50-th min. and reduced the number of rats with tremors from 60 min. after Oxotremorine administration in comparison with the control. At the dose of 10 mg/kg Rapitalamat decreased the severity of tremor in the rats since 30-th min., and the number of rats in the group with the tremor signs since 50-th min. and further on. In the group of rats which were administered with the reference drug Levodopa at the dose of 50 mg/kg, the severity of tremor was significantly reduced since 50-th min. after Oxotremorine administration, and a significant decrease in the % of rats with tremorsin this groupwas observed 60 min. after Oxotremorine administration.

DISCUSSION

Rapitalam decreased the severity of Oxotremorine-induced tremor in rats due to the pronounced anticholinergic activity. To explain the anticholinergic activity of Rapitalam, it is necessary to consider the interaction mechanisms in the extrapyramidal system of the brain in detail. The extrapyramidal system is a set of brain structures involved in the management of movements, maintenance of muscle tone and posture. This system can be represented in three major formations: paleostriatum (globus pallidus), neostriatum (caudate nucleus and putamen) substantia nigra [13]. With the normal functioning of the extrapyramidal system, cholinergic motor neurons of the spinal cord are in a state of constant activity and increase muscle tone. GABAergic neurons of globus pallidus inhibit motor neurons of the spinal cord, which leads to a decrease in the muscle tone. In parallel, GABAergic neurons of the caudate nucleus inhibit globus pallidus, therefore, inhibition of motor neurons of the spinal cord stops, and the muscle tone increases. The adequate control of the muscle tone in the extrapyramidal system is carried out by the interaction of excitatory glutamatergic neurons of the cortex, inhibitory dopaminergic neurons of substantia nigra and excitatory cholinergic neurons of the caudate nucleus (Fig. 1) [14, 15].

The pathogenesis of Parkinson's disease is based on

the death of dopaminergic neurons of substantia nigra, which leads to the increase in the tone of cholinergic neurons of the caudate nucleus. Under the influence of glutamatergic neurons of the cortex, stimulation of the cholinergic neurons of the caudate nucleus occurs and those, in turn, support GABAergic neurons in the active state. Since the caudate nucleus constantly inhibits globus pallidus, the latter does not have an inhibitory effect on motor neurons and the muscle tone remains high [16].

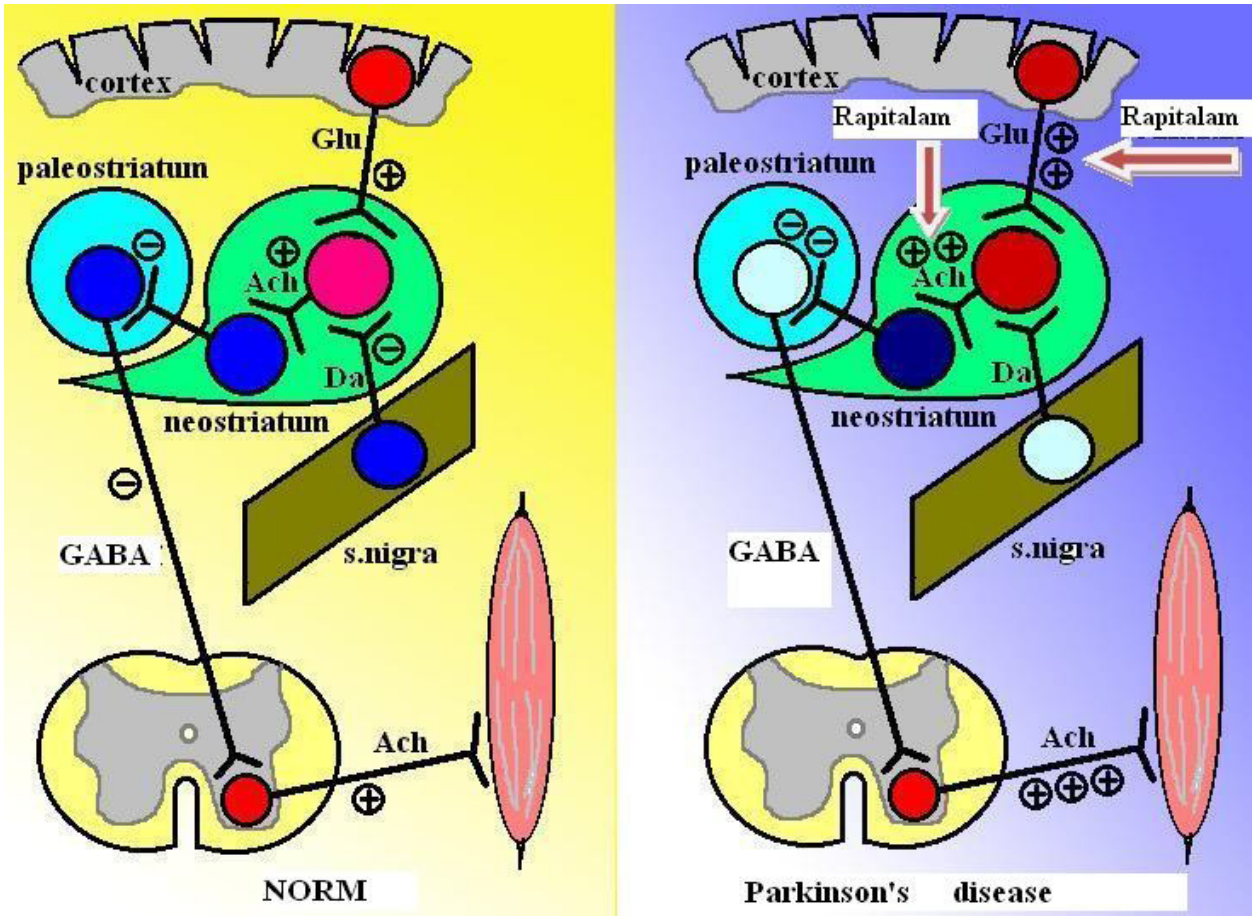

Figure 1 – Biological targets of Rapitalam in Parkinson's disease [16].

Note: The normal relationships between the components of the extrapyramidal system are on the left. The picture that takes place in a patient with Parkinson's disease is on the right).

Ach – acetylcholine, Glu – glutamic acid, Da – dopamine.

As Fig. 1 shows, Rapitalam suppresses glutamatergic neurons of the cortex and therefore inhibits cholinergic neurons of the neostriatumdue to the reduced stimulating effect of the thalamus. [17, 18]. Thus, under the influence of Rapitalam, there is a decrease in the acetylcholine level, as 1–2% of striatum neurons are represented by acetylcholine–containing interneurons [19], which causes an anticholinergic action of Rapitalam.

In this regard, in the long term, Rapitalam can solve the problem of treating tremor in Parkinson's disease at its early stages.

CONCLUSION

At the dose of 1 mg/kg, Rapitalam does not have a significant effect on the manifestations of tremor in rats. Rapitalam (3 and 10 mg/kg) and Levodopa (50 mg/kg) have a comparable anti-tremor efficacy in rats. Rapitalam at the dose of 10 mg/kg reduces the symptoms of Oxotremorine-induced tremor in comparison with the control group in a shorter period of time than Levodopa.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

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