ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Anti-Inflammatory Activity of Phosphonates Based on the Model of Chronic Autoimmune Inflammation of the Paws of Rats.

Ildaria Valeeva [1], Albina Titarenko[2,3]*, Veronika Khaziakhmetova[2,3], and Liliya Ziganshina[3].

[1]FSBEI HE Kazan State Medical University MH of the RF,49, Butlerov Str., 420012, Kazan.
[2]Academic Teaching Hospital, Kazan Federal University 420043, Kazan, 18, Volkov Str.
[3]Institute of Fundamental Medicine an Biology? Kazan Federal University, 18, Kremlyovskaya Str.,420008, Kazan

ABSTRACT

Currently, there is an ongoing search for new pharmacological agents capable of exhibiting anti-inflammatory properties in the treatment of chronic autoimmune inflammatory diseases of connective tissue of the movable joints, accompanied by progressive violation of motility of the joints, edema and severe pain, developing joint destruction, disability and disability, particularly in rheumatoid arthritis. Based on white laboratory rats experiments in the modeling of chronic autoimmune inflammation of the paws, similar to the clinical course of chronic autoimmune inflammation of the joints of the human by administering Freund's adjuvant the anti-inflammatory effect of dimephosphone, mephopran, ksidiphone has been evaluated. It has been shown that dimephosphone has an anti-inflammatory effect on the model of chronic autoimmune inflammation of rat paws caused by the administration of Freund's adjuvant. Dimethyl ether of 2-carbometoksipropilphosphonic acid (mephoprane) model of adjuvant arthritis shows inflammatory effect, only when the secondary arthritis, induced by administration of Freund's adjuvant. Ksidiphon has no anti-inflammatory effect, no effect on the rat paw edema, induced by administration of Freund's adjuvant. The results are a prerequisite for carrying out further experimental and clinical studies on the effect of anti-inflammatory mechanisms of phosphonates.

Keywords: Freund's adjuvant, dimephosphone, monophosphonate - mephopran (dimethyl ether of 2-carbometoksipropilphosphonic acid) ksidiphone (etidronate), prednisolone

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Rheumatoid arthritis (RA) - a chronic autoimmune inflammatory disease of the connective tissue of the joints with advanced mobility violation of the joints, swelling, and their strong pain, developing joint destruction, incapacitation and disabilement. The search continues for new pharmacological agents capable of exhibiting anti-inflammatory properties. Experimentally developed model of adjuvant arthritis (AdA) in rats is similar to the clinical course of chronic autoimmune inflammation of the joints, developing with rheumatoid arthritis in humans, and, is, therefore, used in experimental studies evaluating the anti-inflammatory activity of potential drugs [8, 9]. Promising in this respect are the biologically active compounds of di- and monophosphonic series. Previously, anti-inflammatory activity of dimephosphone was demonstrated experimentally on various models of acute inflammation [2, 3, 4].

The purpose of this work is assessment of anti-inflammatory activity of phosphonates: monophosphonatedimephosphone (DMP), its structural analogue - mephopran (MP), diphosphonateksidiphone (KS) On the model of chronic autoimmune inflammation of the paws of rats induced by administration of Freund's adjuvant.

MATERIALS AND RESEARCH METHODS

The experiments were conducted on 40 white mongrel adult laboratory rats of both sexes, weighing 180-200 g, they were divided into 5 groups of 8 rats (4 males and 4 females), kept under standard vivarium conditions. Animal experiments were performed in strict compliance with the requirements of the European Convention 'On Protection of vertebrate animals used for experimental and other scientific purposes" [Strasbourg, 1986] and "The rules of good laboratory practice in the Russian Federation" (the order of the Ministry of Health of the Russian Federation dated 23.08.2010 № 708n g) [7].

The development of adjuvant arthritis in rats was induced by administration plantar aponeurosis of the left hind paw of 0,1 ml of Freund's adjuvant (Sigma).

Before the experiment, and for 40 days with an interval of 4-5 days, we evaluated the dynamics of body weight and the intensity of the primary arthritis in the degree of growth of the left leg, and secondary arthritis - to change the volume of the contralateral (right) paw of rats on the same terms of research. The intensity of the formation of abnormal process in the animals was determined by plethysmometer UgoBasile on the difference of volume of paws before the administration of Freund's adjuvant, and on 3, 7, 11, 15, 20, 27, 31, 38, 41 days after its introduction (the researcher who performeds measurements of volume of rat paw with plethysmometer was "blinded" concerning the rats belonging to a particular study group). The degree of paw swelling was expressed in % of increase to their original volume. Since administration of adjuvant, the rats of the experimental groups were administered into the stomach by gavage the studied drugs in equimolar doses, corresponding to 1 mM / kg of the body weight per day: monophosphonate dimephosphone - 208 mg / kg [5] mephopran - 210 mg / kg [6] diphosphonate -ksidiphon 45 mg / kg [1], a glucocorticoid prednisolone at a dose of 1 mg / kg of the body weight of rats. The rats of control group were injected daily intragastrically with distilled water at 1 ml / 100 g of the body weight.

The research results were processed statistically using the Student's t-test and the nonparametric method of Fisher's method and presented as M + m (M-average value, m-standard error of the arithmetic mean). Differences were taken for significant at a probability level of 95% or greater ($p \leq 0,05$).

RESULTS AND THEIR DISCUSSION

Experimental data are presented in Table 1 and Table 2.

The evaluation results of animal body weight throughout the experiment after administration of Freund's adjuvant, dimephosphone, mephopran, ksidiphone and prednisolone are given in Table 1.

ISSN: 0975-8585

Table 1. Rat body weight growth after administration of Freund's adjuvant and the test compounds (orally), in% of the initial values, m±M

Groups	Indices							
	Before administration of adjuvant, %	The days of measurements of body weight of rats after being injected with Freund's adjuvant						
		7	11	16	23	29	35	41
Control (water)	100%	99%	100%	102%	102%	105%	104%	105%
Dimephospho 1 мМ/kg	100%	87%	100%	102%	106%	101%	101%	100%
Mephopran 1мМ/kg	100%	103%	99%	103%	103%	102%	104%	106%
Ksidiphon 45 mg/kg	100%	93%	95%	99%	97%	95%	92%	91%
Prednisolone 1 мМ/kg	100%	101%	102%	105%	106%	103%	104%	103%

When assessing the of body weight of the rats in dynamics between the experimental and control groups, significant differences were not found (Table 1).

The primary reaction to the introduction of Freund's adjuvant (swelling of the ankle joint of the left hind paw) was observed in 24 hours after propagation of the model as signs of inflammation of the limb, the intensity of which is quantified by the volume of paw edema (Table 2).

On the 11th day after administration of adjuvant, 20% of the animals had other limb edema (ankle swelling right (intact) hind leg and ankle joints of the front paw) developed - secondary arthritis (Table 2).

Introduction of dimephosphon helped to reduce the swelling of the left paw of the rats on the 3d day of the study by 22% (p=0,05), on the 7th day - 33%(p=0,05),the 11th day – 36%(p<0,05), the 15th day 33%(p<0,05), the 20th day – 42%(p<0,05), on the 27th - 50% (p=0,05),on the 31st – 45% (p<0,05),the 38th - 49% (p<0,05) and on 41st day - 55% (p=0,05) (table 2).

On the 11th day of the experiment, there was a tendency to decrease swelling of the right paw (secondary arthritis) by 70% (p <0.1), by the 20th day - a significant decrease in puffiness by 90% (p <0.05) relative to the figures of the animals of the control group. Swelling of the right paw of the animals that were injected with mephopran disappeared after the 20th day of the experiment (Table 2)

Introduction of diphosphonate- ksidiphon did not affect the intensity of the development of both primary and secondary arthritis at all test stages (Table 2).

Prednisolone reduced swelling of the left paw of the rats on the 3d day of the study by 21% (p = 0.05), on the 7th day - 32% (p <0.05), the 11th day - 31% (p <0 , 05). From 15th to 30th days of the experiment a significant anti-inflammatory effect of prednisolone was not observed. On day 31 of administration of prednisolone against the background of Freund's adjuvant, edema of the left paw of the rats significantly decreased by 25% (p <0.05) on day 41 - 27% (p = 0.1) as compared with the control group.

ISSN: 0975-8585

Table 2. Indices of growth of rat paw after injection of Freund's adjuvant and the test compounds (in), in% to the initial values, M±m

Groups	Dates of registration changes in the volume of rat paw								
	3d - day L. p.	7th day L.p.	11th day L.p. R. p.	15th day L. p. R.p..	20th day L.p. R.p.	27th day L. p. R.p..	31st day L. p. R.p..	38th day L. p. R.p..	41st day L. p. R.p...
Control (water)	86,8±8,4 100%	88,0±8,2 100%	87,2±8,0 100% 17,2±5,9	87,9±6,8 100% 10,0±3,3	83,1±7,1 100% 14,2±4,5	54,3±,7,8 100% 6,8±3,0	46,5±5,2 100% 5,6±2,6	49,4±4,0 100% 7,5±4,9	52,8±14,0 100% 2,5±1,9
Dimephosphone 1 мМ/kg	68,1±6,4 ** 78,4%	58,0±8, 2** 66%	55,6±4, 7* 64% 6,96±4,1	59,3±4, 4* 67% 7,45±3,4	47,8±11, 5* 58% 3,6±1,9*	27,1±7, 1** 50% 3,7±2,2	26,1±3, 9* 55% 2,8±1,8	25,1±3, 6* 51% 0,42±0,3 *	23,9±3,1 ** 45% 2,8±2,0
Mephopran 1 мМ/kg	89,9±13, 2 104%	69,8±9, 0 80%	81,4±10 ,7 93% 5,1±3,3* *	86,7±9, 6 99% 6,3±3,7	69,1±9,7 83% 1,3±1,2*	37,5±6, 4 69% 0	28,7±7, 0 62% 0	31,3±10 ,2 63% 0	36,4±8,8 69% 0
Ksidiphone 45 мг/kg	91,4±18, 4 105%	81,2⍰11 ,9 92%	81,2⍰12 ,0 93% 15,0⍰3,6	75,9⍰14 ,6 86% 17,7⍰3,7	68,8⍰8,6 83% 8,85⍰4,0	50,7⍰13 ,6 94% 2,85⍰1,3 *	75,9⍰2 4,0 166% 4,1⍰2,3	58,7⍰14 ,8 120% 3,9⍰2,2	58,9⍰14, 6 113% 8,0⍰4,4
Prednisolone 1 мг/kg	68,5±8,0 ** 79%	60,5±4, 8* 68%	59,9±4, 5* 69% 11,2±3,9	89,6±7, 6 102% 15,8±6,0	69,3±8,3 84% 27,2±11,9	52,3±10 ,3 96% 13,5±10, 4	35,5±4, 0 76% 7,5±4,7	37,1±2, 4* 75% 5,7 ±3,5	38,6±2,0 * 73% 9,6±4,4

Note: * - p<0,05 as compared to control;
** - 0,05 < p< 0,1 as compared to control;
Left paw – L.p., eight paw – R.p.

Thus, based on the model of chronic autoimmune inflammation of rat paws caused by the administration of Freund's adjuvant, monophosphonates - dimephosphone and mephopran as well as prednisolone exhibited anti-inflammatory action, at the same time, anti-inflammatory activity of mephopran was manifested only in the secondary arthritis. Ksidiphon did not have anti-inflammatory action.

Earlier, we showed in the experiments on the rats on a model of indomethacin-induced gastropathy in prophylactic administration phosphonates of dimephosphone and ksidiphon that dimephosphone, unlike ksidifon, reduced the incidence of erosive ulcers caused by non-steroidal anti-inflammatory agents, and exhibited the antioxidant effect [2]. Therefore, it suggests that to realize the anti-inflammatory action of test phosphonates, several mechanisms are involved [4].

Our results are a prerequisite to conduct further experimental and clinical examinations on the effect of anti-inflammatory mechanisms of phosphonates.

SUMMARY

- Dimephosphon exhibits anti-inflammatory effect on the models of chronic autoimmune inflammation of the paws of rats induced by administration of Freund's adjuvant.
- Dimethyl ether of 2-carbometoksipropilphosphonic acid (mephopran) shows inflammatory effect on the model of adjuvant arthritis only when the secondary arthritis induced by administration of Freund's adjuvant.

- Ksidiphon has no effect on swelling of the rat paws, induced by administration of Freund's adjuvant.

ACKNOWLEDGEMENTS

The work is performed in the Central Research Laboratory, Kazan State Medical University, and in the Kazan Federal University, including according to the Russian Government Program of Competitive Growth of Kazan Federal University.

REFERENCES

[1] Alekseeva N.V., Yurieva E. A., Balandin E. K. et al. Current Issues in Pediatric Pharmacotherapy, Moscow, 1982.- P. 111-115.
[2] Valeeva I.K., Titarenko A. F. Ziganshina, L. E. Experimental and Clinical Pharmacology.- 2010.- № 12, P.21-24.
[3] Valeeva I. K., Titarenko A. F. Khaziakhmetova V. N., Ziganshina L. E. Experimental and Clinical Pharmacology.- 2011.- № 3 , P.13-16.
[4] Ziganshina, L. E., Studentsova I. A., Valeeva I. K., Ziganshin A. U. Experimental and Clinical Pharmacology. - 1992.- №2.- P. 43-45.
[5] Ziganshina, L. E, Burnashova Z.A., Valeeva I. K., et al. Experimental and Clinical Pharmacology.- 2000.- № 6 , P.39-42.
[6] Ismagilov V. S. Comparative Evaluation of Biological Activity of Alkylphosphonic Acids. Thesis for Candidate of Medicine.- Kazan. 1991. 140p.
[7] Approval of the Rules of Laboratory Practice: Order of the RF Ministry of Public Health of August 23 2010. N 708n: Registered in Ministry of Justice of RF of October13. N 18713 // Rus.Gas. 2010. October 22.
[8] Trinus F. P., Klebanov B. M., Kondratyuk V. I. et al. Guidelines on Experimental (Preclinical) Study of Non-Steroidal Anti-Inflammatory Pharmacological Substances.- M.- 1983.- 16p.;
[9] Kahlenberg J.M., Fox D.A. Advances in the Medical Treatment of Rheumatoid Arthritis Hand Clin. – 2011. 27(1). – pp.11–20.