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The Study of The Dependence of Phlogotropic And Membrane-Tropic Activity of Phosphonates on Their Chemical Structure.

Veronika Khaziakhmetova[1,2]*, Daniyar Khaziakhmetov[3], Ayrat Ziganshin[3], and Liliya Ziganshina[1].

[1]Kazan Federal University, Institute of Fundamental Medicine and Biology, Kremlevskaya Str. 18, Kazan, 420008
[2]University Clinic, Kazan Federal University, Volkova Str. 18, Kazan, 420043
[3]Kazan State Medical University, Butlerova Str. 49, Kazan, 420012

Abstract

This paper deals with the study of the anti-inflammatory and membranotropic activity of dimephosphone, mephoprane and new chemical synthesis products belonging to different series of substituted phosphonic acid derivatives - monophosphonates. The effect of monophosphonates on the intensity of carrageenin inflammation in mice and rats was studied. The relationship of chemical structure and membrane-protective activity was analyzed on the models of osmotic and free-radical hemolysis. It was established that the organophosphorousc compounds - derivatives of alkylphosphonic acids - show anti-inflammatory and membrane-tropic activity. The anti-inflammatory and membrane-tropic activity of functionally substituted monophosphonates depends on the length of the hydrocarbon radicals in the ester fragments of the molecule: the greater length and the presence of the methyl radicals and a carbonyl (carboxyl) group in the alkyl fragment of the molecule provide greater activity. The greatest activity has a 2-carbobutoxypropyl-phosphonic acid dibutyl ether - ephorane (IIId).

Keywords: phosphonates, dimephosphone, ephorane, carrageenin, inflammation, free radical hemolysis, red blood cell osmotic hemolysis, membrane-tropic activity, rats, mice.

Corresponding author

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INTRODUCTION

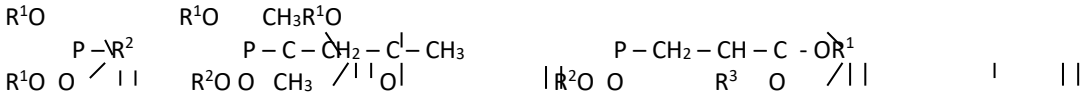
New approaches to pharmacological regulation of inflammation, a change of the philosophy of searching for anti-inflammatory drugs [3], the imperfection of the available antiphlogistics in practical medicine have identified an increased interest in studying the potential phlogotropic agents among various new classes of chemical compounds. Promising compounds in this regard are those of monophosphonic series [6,7].

This paper deals with the study of the original medicinal drugs developed in Kazan - dimephosphone, mephoprane and new chemical synthesis products belonging to different series of substituted phosphonic acid derivatives - monophosphonates. The ground for in-depth study of their phlogotropic activity was the earlier established anti-inflammatory activity of dimephosphone [1,2].

MATERIALS AND METHODS

The substances of the tested compounds were synthesized in the Technological Laboratory of A.E. Arbuzov Institute of Organic and Physical Chemistry, the Russian Academy of Sciences (RAS) under the guidance of A.O. Vizel.

The chemical structure of the tested phosphonates is as follows:



I IIa – г IIIa – е

I: R^1 = H, R^2 = C_4H_9
IIa – г: а, R^1=R^2=Na; б, R^1=R^2=H; в, R^1=CH_3, R^2=H; г, R^1=R^2= CH_3;
IIIa – е: а, R^1=R^2 = CH_3, R^3=H; б, R^1=R^2=R^3 = CH_3; в, R^1=R^2=C_2H_5, R^3=H; г, R^1=R^2=C_4H_9, R^3=CH_3; д, R^1=Na, R^2=H, R^3=CH_3; е, R^1=R^2=H, R^3=CH_3.

The anti-inflammatory activity (AIA) was studied on adult outbred albino mice weighing 18-20 g and outbred laboratory albino rats weighing 150-230 g of both sexes, using carrageenin (1%) as phlogogenic agent, which was introduced subplantarly in the left hind paw in a volume of 0.05 ml to mice and 0.1 ml to rats [4,5]. The size of edema was determined with the use of plethysmometer Ugo Basile / Italy / on the difference in paw volume before the introduction of the pro-inflammatory agent and 3 hours after its administration. All tested compounds were administered at a dose of 1 mM/kg intraperitoneally twice - 15 minutes before and one hour after the simulation of paw inflammation. A control drug - acetylsalicylic acid (ASA) (Tatkhimpharmpreparaty, Russia) was administered intragastrically at a dose of 100 mg/kg - 15 minutes before and one hour after the simulation of paw inflammation.

The experimental animals were kept in vivarium conditions (with the natural lighting regime; temperature - 22-24°C; relative humidity - 40-50%) on a standard diet (GOST R 50258-92) [9]. Studies were carried out in accordance with the rules of good laboratory practice (GLP) for preclinical research in the Russian Federation [15], as well as the rules and recommendations of the International European Convention for the Protection of Vertebrate Animals used in experimental studies [10]. The study was approved by Local Ethics Committee.

The influence of monophosphonates on resistance of erythrocyte membranes was studied by the method by Inglot A.D., Wolna M. [8]. Erythrocyte suspension was used, prepared from heparinized blood of rats by diluting 0.9% sodium chloride with sodium phosphate buffer. When simulating the osmotic hemolysis, hypotonic medium was created with the use of saline solution containing 55-57 mM sodium chloride. To generate the free radicals, a Fenton reagent was used, which is a mixture of iron sulfate (II) and hydrogen peroxide. 1% suspension of red blood cells was incubated with the test compounds at concentrations $1x10^{-9}$M - $1x10^{-1}$ M at room temperature for 30 minutes, then Fenton reagent was added. After 24 hours, after adding

the Fenton reagent, the suspension was centrifuged - 1200 rev/min for 15 minutes. The intensity of hemolysis was spectrophotometrically considered at a wavelength of 543 nm, and by the content of hemoglobin in the supernatant. The control (100%) were samples free of phosphonates. Simultaneously, 4-8 parallel samples were studied.

Data from all experiments were statistically processed using the Student's t-test and presented as M±m (M - average value, m - standard error of the mean).Differences were considered significant at a probability level of 95% or greater ($p \leq 0.05$).

RESULTS AND DISCUSSION

The studied compounds - derivatives of alkylphosphonic acids were arbitrarily divided into butilphosphonic acid derivatives (I, II a-d) and phosphonic acid derivatives containing a carboxyl group (III a-e).

Studying the effect of phosphonates on the intensity of the carrageenin inflammation we have found that the greatest anti-inflammatory activity is shown by compound IIb, reducing thereby the size of paw edema in mice by more than half (54%), in rats - by more than 5 times (by 84%), and a compound IIId that reduces the intensity of the carrageenan paw edema in mice by more than half (52%), and in rats - by more than five times (82%). (Table 1). Such a high anti-inflammatory activity of the compound IIId at a dose of 1 mM/kg caused the expediency of assessing the dependence of its effectiveness on the dose. The tests of doses of 1/16 mM/kg to 1 mM/kg have established a dose-dependent anti-inflammatory activity of the compound IIId. Its average effective dose (ED_{50}) was 150 mg/kg (0.45 mM/kg). These results substantiate the dependence of anti-inflammatory activity in a series of derivatives of alkylphosphonic acids on the lipophilic compounds and on the length of hydrocarbon radicals in ester groups: a long length provides greater anti-inflammatory activity. It has been also shown the importance of the presence of a molecule of methyl and carbonyl group in the alkyl fragment for the manifestation of phlogotropic properties.

The protective effect of the compounds I, IIa, IIb, IIIe intensified during osmotic hemolysis with increase in their final concentrations in the incubation medium. Maximum prevention of the compound hemolysis was induced at a concentration of 10^{-1} M. Thus, the experiments confirm the correctness of theoretical calculations of the isotonic agents concentration [11], and indicate that the protection of erythrocytes during the action of these compounds is obviously osmotic. Compound IId, IIId reduced the intensity of osmotic hemolysis of rat erythrocytes without a clear dependence of the acting force on the changing concentration, thus, their membrane-protective effect is not related to osmotic protection of erythrocytes, but is a reflection of true interaction of substances with the membrane components, determining its resistance to the damaging effects of hypotonic medium. (Tables 2, 3).

Analyzing the chemical structure - membrane-ptotective activity relationship in a number of derivatives of buthylphosphonic acid on the background of free-radical damage of erythrocyte membranes, we can distinguish the dependence of effects on the modification of the chemical structure of the phosphonate fragment of the molecule. (Table 4). The absence of the substituent groups and keto-group therein - compound I - defines the hemolysis-stimulating effect. Changes in the effect direction during increase in the concentration of the compound may reflect its interaction with the participants of free-radical reactions. Compound IId differs from the compound I in having two methyl substituents inn the first carbon atom in the phosphonate fragment of the molecule. This is, apparently, the thing that determines its protective effect, based on a direct interaction with the structural components of the membrane. Considering the dependence of the action of the phosphonic acids containing a carboxyl group (compounds IIIb, IIIc, IIId) on the length of a hydrocarbon radical in the ester fragments of the molecule, we can note the following: the longer the hydrocarbon radical is, the lower the concentration and the greater the intensity are, at which and with which the compound shows its ability of stimulating the free-radical hemolysis. (Table 5). The dependence of the stimulating effect of the compounds on the concentration indicates their direct interaction with free radicals.

Analysis of the ratio of anti-inflammatory and membrane-protective activity of the studied compounds shows their clear parallelism for the most effective anti-inflammatory compounds, which are not acids. Both of these actions are characteristic of the compound IIId - 2-carbobutoxypropyl-phosphonic acid dibutyl

ether (ephorane). This confirms the statement on the interdependence of the anti-inflammatory and membrane-stabilizing effect of non-steroidal anti-inflammatory drugs [8].

Thus, it was established that the organophosphorousc compounds - derivatives of alkylphosphonic acids - show anti-inflammatory and membrane-tropic activity. The anti-inflammatory and membrane-tropic activity of functionally substituted phosphonates depends on the length of the hydrocarbon radicals in the ester fragments of the molecule: the greater length and the presence of the methyl radicals and a carbonyl (carboxyl) group in the alkyl fragment of the molecule provide greater activity. The greatest activity has a 2-carbobutoxypropyl-phosphonic acid dibutyl ether - ephorane (IIId).

Table 1: The effect of monophosphonates on the intensity of paw edema in rats and mice, caused by subplantar introduction of carrageenin (1%)

Compound	Empirical formula	Dose, mg/kg	% of edema depression as compared with control 3 hours after introduction of carrageenin	
			mice (n=7)	rats (n=7)
I	$C_6H_{15}PO_3$	166	-	-
II a	$C_6H_{11}PO_4\ Na_2$	224	22*	-
II b	$C_6H_{13}PO_4$	180	32*	56*
II c	$C_7H_{15}PO_4$	194	54*	80*
II d	$C_8H_{17}PO_4$	208	23*	24*
III a	$C_6H_{13}PO_5$	196	-	36*
III b	$C_7H_{15}PO_5$	210	-	22*
III c	$C_9H_{19}PO_5$	238	20*	56*
III d	$C_{16}H_{33}PO_5$	336	52*	82*
III e	$C_4H_7PO_5Na_2$	212	-	-
III f	$C_4H_9PO_5$	168	37*	76*
Acetylsalicylic acid (ASA)		100	-	53*

Note: * - $P<0.05$ as compared with control.

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Table 2: The effect of butylphosphonic acid derivatives on osmotic hemolysis of rat erythrocytes (% to control, M±m)

Sample concentration of compounds (M)	I($C_6H_{15}PO_3$)	II a ($C_6H_{11}PO_4Na_2$)	II b ($C_6H_{13}PO_4$)	II c ($C_7H_{15}PO_4$)	II d ($C_8H_{17}PO_4$) (dimephosphone)
Control	100±2	100±2	100±5	100±5	100±1
10^{-9}	94±2	103±4	99±5	103±5	100±2
10^{-8}	97±1	100±1	97±3	107±7	100±3
10^{-7}	94±1*	99±3	99±4	94±4	98±2
10^{-6}	91±3⁰	101±0.3	88±2	93±5	100±1
10^{-5}	91±5	98±5	90±5	93±4	86±3*
10^{-4}	96±1	105±2	99±1	95±3	80±6*
10^{-3}	96±4	91±2*	87±3	91±7	90±4*
10^{-2}	91±2*	30±1*	85±23	94±5	95±2*
10^{-1}	84±4*	5±0.5*	48±8	31±4*	107±7
Isotonic concentration	$2.9x10^{-1}$ M (4.8%)	$1.16x10^{-1}$ M (2.6%)	$2.6x10^{-1}$ M (4.7%)	$2.6x10^{-1}$ M (5.1%)	$2.9x10^{-1}$M (6%)

Note: * - P<0.05 as compared with control.

Table 3:The effect of phosphonic acid derivatives containing carboxyl group on osmotic hemolysis of rat erythrocytes (% to control, M±m)

End concentration of compounds (M)	III a ($C_6H_{13}PO_5$)	III b ($C_7H_{15}PO_5$) (mephoprane)	III c ($C_9H_{19}PO_5$)	III d ($C_{16}H_{33}PO_5$) (ephorane)	III e ($C_4H_7PO_5Na_2$)	III
Control	100±2	100±11	100±1	100±2	100±5	
10^{-9}	97±2	107±8	95±3	83±3*	101±4	
10^{-8}	98±2	115±10	100±1	79±4*	99±2	
10^{-7}	95±3	103±4	98±1	89±3*	93±10	
10^{-6}	99±2	112±9	97±1	79±3*	103±5	
10^{-5}	98±3	110±14	99±1	80±2*	99±2	
10^{-4}	101±1	124±19	101±4	87±0.4*	103±3	
10^{-3}	98±2	121±8⁰	102±2	…^	95±3	
10^{-2}	97±1	105±6	99±1	…	74±3*	
10^{-1}	101±3	153±19*	85±1*	…	5±1*	
Isotonic concentration	$2.9x10^{-1}$ M (5.7%)	$2.9x10^{-1}$ M (6.1%)	$2.9x10^{-1}$ M (6.9%)	$2.9x10^{-1}$ M (9.7%)	$1.16x10^{-1}$ M (2.5%)	

Note: * - P<0.05 as compared with control.

^ - study of the effect of high concentrations of the compound is impossible due to its low solubility in water.

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Table 4: The effect of butylphosphonic acid derivatives on free-radical hemolysis of rat erythrocytes (% to control, M±m)

Sample concen-tration of the drug (M)	I($C_6H_{15}PO_3$)	II a ($C_6H_{11}PO_4Na_2$)	II b ($C_6H_{13}PO_4$)	II c ($C_7H_{15}PO_4$)	II d ($C_8H_{17}PO_4$) (dimephosphone)
Control	100±2	100±2	100±5	100±10	100±1
10^{-9}	203±14*	79±3	124±17	111±13	63±12*
10^{-8}	171±7*	71±9	133±25	114±19	65±12*
10^{-7}	133±15	102±15	144±32	117±36	71±13
10^{-6}	166±15*	85±18	142±23	111±32	52±8*
10^{-5}	146±11*	78±7	147±24	115±12	94±4
10^{-4}	149±11*	85±18	136±17	125±43	111±4
10^{-3}	151±15*	200±12*	151±18	137±24	128±3*
10^{-2}	118±32	53±1*	146±13	88±15	93±8
10^{-1}	38±11*	54±3*	68±5*	56±6*	78±8*

Note: * - P<0.05 as compared with control.

Table 5: The effect of phosphonic acid derivatives containing carboxyl group on free-radical hemolysis of rat erythrocytes (% to control, M±m)

Sample concen-tration of the drug (M)	III a ($C_6H_{13}PO_5$)	III b ($C_7H_{15}PO_5$) (mephoprane)	III c ($C_9H_{19}PO_5$)	III d ($C_{16}H_{33}PO_5$) (ephorane)	III e ($C_4H_7PO_5Na_2$)	III f ($C_4H_9PO_5$)
Control	100±2	100±10	100±11	100±15	100±10	100±9
10^{-9}	126±8	105±2	98±19	143±21	96±8	99±7
10^{-8}	126±5	73±7	109±23	169±18*	94±16	81±16
10^{-7}	128±4	74±5	108±12	168±18*	104±43	100±17
10^{-6}	119±4	67±13	103±10	269±66*	88±37	67±13
10^{-5}	96±11	59±6*	146±16*	281±62*	80±6	93±11
10^{-4}	109±7	67±6*	157±27*	519±70*	97±7	63±9*
10^{-3}	116±4	61±8*	261±29*	…^	120±9	31±7*
10^{-2}	104±4	70±3*	360±45*	…	83±26	17±2*
10^{-1}	83±13	335±58*	354±50*	…	73±4*	101±7

Note: * - P<0.05 as compared with control.

^ - study of the effect of high concentrations of the compound is impossible due to its low solubility in water.

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