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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Synthesis and Anti-influenza Activity of Vinylphosphonic Acid (Co)polymers

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Abstract—New copolymers of vinylphosphonic acid (VPA) with 2-deoxy-2-methacrylamido-D-glucose, 4-acryloylmorpholine (4-AM), and acrylamide have been synthesized, and their antiviral activity against influenza virus has been studied in in vitro and in vivo experiments. Optimal antiviral characteristics and low cytotoxicity were exhibited by the copolymer of VPA with 4-AM, composition 56 : 44 mol %, molecular weight 33000. The polymer exhibited virus-inhibiting properties with an IC_{50} = 1 μg/mL and a selectivity index of 302. Prophylactic intranasal administration of the polymer in a murine model of influenza pneumonia completely prevented the virus-induced death of animals, whereas the level of mortality in the placebo group was 90%. The results of this study indicate a high antiviral potential of polymeric compounds based on VPA.

Keywords: phosphorus-containing polymers, vinyl phosphonic acid, copolymers, influenza, antiviral compounds

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Influenza, despite the obvious advances in the field of vaccinology and chemotherapy, continues to be a difficult-to-control infection. This is due to the peculiarities of the pathogen itself, primarily, its high rate of evolution, which leads to evasion of the immune response and the formation of drug-resistant mutants [1]. Therefore, along with the specific therapy, pathogenetic drugs aimed at suppressing reactive processes, such as cytokine storm, inflammatory infiltration of target organ tissues, etc., are used [2]. In this regard, the search for new antiviral drugs, especially nonspecific and effective against viruses of different antigenic groups, is a priority for medical science.

Polymeric synthetic compounds exhibit a wide range of biological activity, including that against a wide variety of groups of viruses. For example, the activity of sulfated polysaccharides from seaweeds, such as galactan sulfate, sulfated xylomannan, carrageenan, etc., against adenovirus, influenza virus, respiratory syncytial virus, coronavirus, etc., was shown [3]. Phosphate-containing polymers are synthesized in cells and are one of the factors of antiviral protection, in particular, against the SARS-CoV-2 virus [4]. Synthetic polyphosphates against coronavirus [5, 6] and against HIV [7, 8] exhibit a similar activity.

The aim of this study was to synthesize new synthetic polymers based on vinylphosphonic acid (VPA) and to evaluate their antiviral activity against the influenza virus in cell culture experiments and in a model of influenza pneumonia in laboratory animals.

VPA homopolymer and copolymers with 2-deoxy-2-methacrylamido-D-glucose (MAG), 4-acryloylmorpholine (4-AM), and acrylamide (AA) were obtained by free-radical (co)polymerization in sealed glass ampoules in an argon atmosphere for 24 h. In *N*,*N*-dimethylformamide (DMF) or methanol, 2,2'-azobisisobutyronitrile (AIBN) was used as initiator; in aqueous solutions, 2,2'-azobis-(2-methylpropionamidine) dihydrochloride (AMP). The polymers were purified by dialysis against water and isolated by freeze drying.

The composition and structure of the copolymers were determined using ^{1}H and ^{31}P NMR spectroscopy in D_2O solution on a Bruker Avance 400 spectrometer (Germany). In the ^{1}H spectra of all synthesized copolymers, the signals of both comonomers were present; in the ^{31}P spectra, the VPA signal. To determine the composition of copolymers, 2-methacryloyloxyethylphosphorylcholine was used as an external standard as described in [9]. Molecular weights (M_{SD}) were determined by sedimentation and diffusion procedures. Data on the synthesis conditions and properties of the obtained polymers are summarized in Table 1.

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Table 1 Synthesis conditions and characteristics of VPA (co)polymers (M_1)

No.	(Co)polymerization conditions						Characteristics of copolymers		
	$[M_2]$	$[M_1]$: $[M_2]$, mol %	$[M_1+M_2]$, wt %	Solvent	I	[I], wt.% of $[M_1+M_2]$	yield, %	$[M_1]$, mol %	$M_{SD}\times 10^{-3}$
1*	–	100:0	80	H_2O	AMP	1	54	100	30
2	MAG	50:50	10	DMFA	AIBN	2	71	12	20
3	MAG	90:10	10	H_2O	AMP	2	29	53	5
4	4-AM	25:75	20	methanol	AIBN	2	76	13	77
5	4-AM	25:75	20	H_2O	AMP	2	82	14	310
6	4-AM	50:50	20	H_2O	AMP	1	38	56	33
7	AA	25:75	20	DMFA	AIBN	2	93	28	25

24 h, 60°C; I, initiator; * 80°C.

Table 2. Cytotoxicity and antiviral activity of phosphorus-containing polymers against influenza virus A/Puerto Rico/8/34 (H1N1) in MDCK cells

No.	$M_{SD}\times 10^{-3}$	CC_{50}, μg/mL	IC_{50}, μg/mL	SI
1 (VPA/100)	30	>330	1.3 ± 0.2	254
2 (VPA : MAG/12 : 88)	20	>330	280.0 ± 36.0	1.2
3 (VPA : MAG /53 : 47)	5	>330	15.5 ± 2.3	21.2
4 (VPA : 4-AM/13 : 87)	77	30.0 ± 2	>30	<1
5 (VPA : 4-AM/14 : 86)	310	>330	356.0 ± 44.0	<1
6 (VPA : 4-AM/56 : 44)	33	302.0 ± 15.0	1.0 ± 0.2	302
7 (VPA : AA/28 : 72)	25	84.0 ± 6.0	72.0 ± 10.1	1.2

Table 3. Protective activity of phosphor-containing polymer **6** on the murine model of lethal influenza pneumonia

Experimental group	Parameters of lethality			
	mortality (dead/infected)	mortality, %	IP	*p*
Placebo	9/10	90	–	1.0000
6 i/n	0/10	0	100	<0.0001
Incubated virus*	9/10	90	0	0.6813

IP—index of protection, *p*—Mantel–Cox test for pairwise comparison with the placebo group; i/n—intranasal administration of polymer **6** 1 h prior to virus inoculation; *animals were infected with the virus that was preliminarily incubated with polymer **6**.

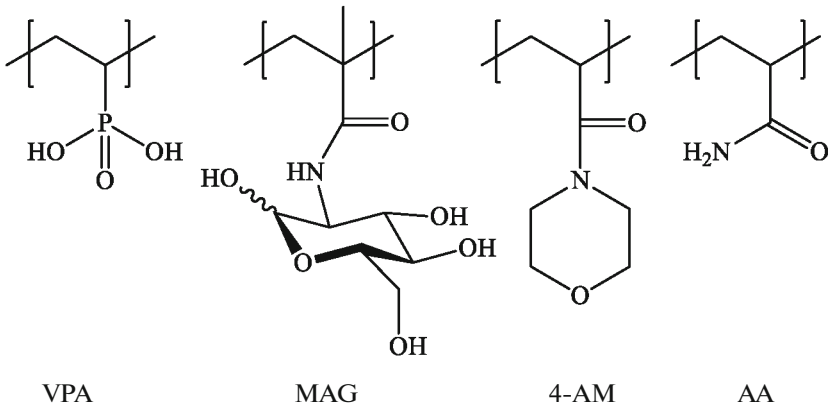
The cytotoxic and antiviral properties of polymers were evaluated in vitro as described in [10].

Outbred female albino mice weighing 16–18 g obtained from the Rappolovo Animal Breeding Facility (Leningrad oblast) were used in the experiments. The polymer aliquots were dissolved in saline and administered to animals (10 animals per group) once intranasally (300 μg/mL, 30 μL per animal) 1 h before infection. Animals in the control group (placebo) received 30 μL of saline.

Mice were infected with the virus at a dose of 5×10^3 $TCID_{50}$ per mouse in a volume of 30 μL intranasally under ether anesthesia. A separate group of animals was infected with the virus at the same dose, but pre-incubated for 1 h with a polymer solution (300 μg/mL). The animals were observed for 14 days. Animal deaths were recorded daily. The significance of differences in deaths of animals was assessed using the Mantel–Cox test in the analysis of Kaplan–Meier survival curves with the use of the GraphPad Prism v.6.01 software package. Differences between groups were considered significant at $p \leqslant 0.05$.

The results of studying the antiviral activity and cytotoxicity of phosphorus-containing polymers in vitro are summarized in Table 2.

Among the compounds analyzed, compound **6** exhibited the highest activity (SI = 302). The biological properties of polymers depended on their chemical



Scheme 1.

composition and molecular weight. For example, the toxicity of polymers **4** and **5**, which have an almost identical ratio of WPA and 4-AM monomers, differed by more than one order of magnitude, with the polymer with a lower molecular weight being more toxic.

The results of studying the dynamics of animal mortality are summarized in Table 3 and are presented in Fig. 1.

The infection of animals with the influenza virus A/Puerto Rico/8/34 (H1N1) led to their mortality starting from day 5 after infection. On day 14 of the experiment, the mortality of animals in the positive control group was 90%. Preincubation of the virus with the studied polymer did not reduce the mortality rates of animals (p = 0.6813), whereas preliminary intranasal administration of mice with the polymer solution resulted in 100% protection against lethal influenza infection ($p < 0.0001$).

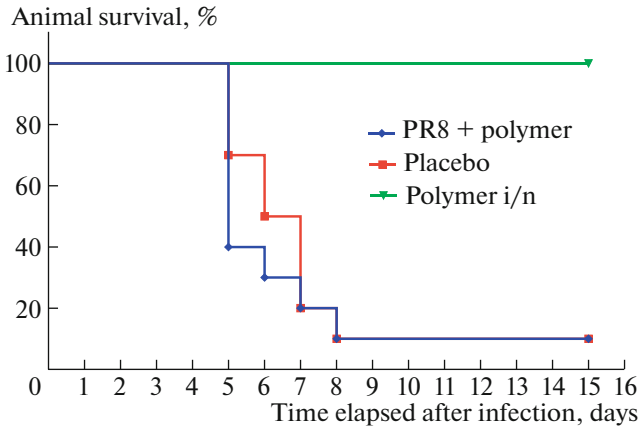


Fig. 1. Kaplan–Meier survival curves for influenza virus A/Puerto Rico (H1N1)-caused pneumonia in mice administered with the polyphosphonic acid-based polymer **6**. Designations: PR8+ polymer—animals were infected with the virus that was preliminarily incubated with polymer **6** (300 μg/mL) for 1 h; Placebo—animals were administered intranasally with saline; polymer i/n—polymer **6** was administered intranasally 1 h prior to infecting.

CONCLUSIONS

We performed the synthesis and studied the properties of copolymers of vinylphosphonic acid (VPA) with 2-deoxy-2-methacrylamido-D-glucose, acrylamide, and 4-acryloylmorpholine (4-AM) as potential drugs inhibiting the infectious activity of the influenza virus. It was shown that the copolymer of VPA with 4-AM in the ratio 56 : 44 with a molecular weight of 33000 has the optimal antiviral characteristics. The polymer had a low cytotoxicity and exhibited virus-inhibiting properties with IC_{50} = 1 μg/mL and a selectivity index of 302. Prophylactic intranasal administration of the polymer in a murine model of influenza pneumonia completely prevented specific mortality, whereas the mortality in the placebo group reached 90%. Previous studies also indicate the ability of phosphorus-containing polymers to activate the nonspecific antiviral defense of the body [11], which may determine the complex mechanism of combating influenza infection when using polymers of the studied composition. The results obtained in this study indicate a high antiviral potential of the polymeric compounds based on vinylphosphonic acid.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. The design of the study was approved by the Ethics Committee of the St. Petersburg Pasteur Institute (protocol no. 7A dated February 15, 2021).

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