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BIOPHYSICS AND MEDICAL PHYSICS

The Physicochemical Basis of the Biological Activity and Pharmacological Properties of the Antiviral Agent Panavir

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Abstract—The agent Panavir possesses versatile antiviral, anti-inflammatory, and analgesic effects, which still have not been explained at the molecular level. Here, we propose a simple physicochemical model of Panavir nanoparticles, which allows one to explain its effects by the mechanical activation of cells, including those of the immune system. Thus, there is no need for binding of the Panavir particles to any supramolecular chiral receptor for manifestation of its all known effects.

Keywords: Panavir, soft nanoparticles, antiviral effect, cell membrane, cytoskeleton, activation of immune cells.

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INTRODUCTION

In recent years polysaccharides of plant origins have attracted the attention of researchers due to confirmed data on their biological activities and pharmacological effects [1, 2]. Information about the mechanisms of action of these agents is very contradictory. In most cases, it is believed that polysaccharides have the properties of adjuvants and may activate immune cells by binding to their receptors [3].

The Russian agent Panavir is among polysaccharide drugs with confirmed antiviral effects [4].

Panavir has been recommended for complex treatment of herpes virus infections of different localizations, secondary immune-deficient states against the background of infectious diseases, cytomegalovirus infection in female patients with habitual miscarriages, papilloma virus infection, tick-borne encephalitis, and rheumatoid arthritis in immune-compromised patients. Panavir is an antiviral drug with a broad spectrum of actions and very low toxicity. Moreover, it exhibits an immunomodulatory effect, increases nonspecific resistance to various infections, and possesses anti-inflammatory and analgesic effects. When first administered, Panavir also increases the resistance of the body to toxic substances, i.e., it is an antidote [4].

The molecular mechanisms of Panavir action are not yet entirely clear; however, judging from the effects listed above, we can assume that they should occur when it interacts with the cells of the immune system. As will be shown below, Panavir is a suspension of hydrogel nanoparticles without mechanical rigidity. This distinguishes Panavir from most other polysaccharide-based agents that exhibit antimicrobial, antiviral, and immunomodulatory effects.

In the present work we analyzed the physicochemical mechanisms of the interaction of Panavir particles with the cell membrane, which allows a qualitative explanation of its biological activity and pharmacological properties.

1. THE BIOLOGICAL ACTIVITIES AND PHARMACOLOGICAL PROPERTIES OF PANAVIR

At therapeutic doses, the drug is nontoxic and well tolerated; the therapeutic dose is $\sim 10^{11}$ particles intravenously. The LD_{50} is no lower than 3000 therapeutic doses (this is a lower boundary because of the technical problems of administration of a large number of doses to an experimental animal). Trials showed that Panavir does not possess mutagenic, teratogenic, carcinogenic, allergenic, and embryotoxic effects. The agent does not affect reproductive functions [4, 5].

The wide range of antiviral activities demonstrates the versatility of the mechanism of Panavir action, which cannot be associated with the blocking of any stage in the lifecycle of a particular virus. Interferons (IFNs) play an important role in protecting the body against viral infection. In clinical trials, Panavir did not affect the interferon level in human blood serum; however, it altered the ability of leukocytes to produce IFNα and IFNγ upon stimulation by inducers of interferon synthesis: the leukocyte interferon

increased by 2.7–3 times (up to concentrations corresponding to therapeutic doses of interferon) [6].

According to reports, Panavir has no in vitro effect on the phenotype of leukocytes, i.e., the expression of surface markers, such as CD4, CD8, and other markers is not changed. T-cell proliferation induced by Panavir and the in vitro induction of proliferation by antibodies were not observed. At the same time, application of Panavir for treatment of patients with atopic dermatitis resulted in a significant decrease in the production of many cytokines (interleukins IL2, IL4, IL5, IL10; interferon IFNγ, and tumor necrosis factor TNFα) and a persistent positive anti-inflammatory effect that is likely to be due to the decreased synthesis of neuropeptides, which ultimately leads to a reduction in lymphocyte homing into the skin. The stronger in vivo effect of Panavir compared with the in vitro effect is explained by the fact that the drug's action is not mediated by a T-cell immune system link [7–9].

Along with antiviral activity and anti-inflammatory action, Panavir exhibits an analgesic effect [10]. In tests on human volunteers, 2–3 min after administration Panavir induced characteristic changes in encephalograms, which persisted for at least 1.5 h [6, 11].

The trial data indicate that all biological effects of Panavir are observed for a period ranging from several seconds to several hours after administration. However, considering the size of Panavir particles (about 200 nm) [12], the great variety of pharmacological effects, and the wide range of times of their manifestation, the molecular mechanism of Panavir action is most likely versatile.

2. THE STRUCTURE OF PANAVIR PARTICLES AND THEIR PHYSICOCHEMICAL PROPERTIES

Panavir is a high-molecular-weight polysaccharide (a purified extract of *Solanum tuberosum* shoots). The composition of Panavir particles is rhamnose (2–10%), arabinose (3–15%), glucose (10–67%), galactose (2–27%), xylose (0.1–3%), mannose (0.1–5%), uronic acids (2–5%), traces of lipids, about 100 types of tryptic peptides, peptide nontryptic fragments, as well as the common plant protein RuBisCo (in total, the peptides and proteins are less than 1%). The elemental composition is phosphorus, about 1%; potassium and calcium, about 0.2% each; and manganese, iron, nickel, copper, and zinc, less than 0.1% each. The absence of the reaction with iodine indicates the cross-linking of hexose polymers, which prevents the formation of clathrates of the channel type that are required for the appearance of the characteristic color in solutions of polysaccharides. After storage for 5 years, the activity of the drug does not change [4, 12].

The solubility at pH 7 and 36°C is 13 g/100 g of buffer solution. The condensed phase of the Panavir particles is formed on the surface of its saturated solution (at a concentration of about 11.4%). Aggregation of particles in bulk was not observed even in a saturated solution [4].

The viscosity of a Panavir solution at all concentrations is not substantially different from the viscosity of water in the temperature range of 20–40°C, which indicates the crucial role of electrostatic and possibly the elastic forces in the interaction of the particles. Panavir particles bear a negative charge. The electrophoretic mobility of the particles is −2.1 and −1.96 $\mu m\ s^{-1}\ V^{-1}$ at concentrations of 0.5 and 1.0%, respectively. As calculated from these data (in the Smoluchowski approximation) the zeta potentials were −26.7 and −25.1 mV, respectively [4, 12].

These values of zeta potentials (less than 30 mV in absolute value) typically correspond to unstable colloidal systems. Therefore, a high aggregate stability of Panavir solutions should be caused by other interactions as well. In particular, this may be the hydration interactions due to the high hydration energy of molecular groups in the hydrogel structure. The absence of aggregation of the Panavir particles indicates that the effects of their induced polarization are small, i.e., the charges are related to the macromolecular functional groups [13, 14].

Panavir particles have a spherical shape and are almost a monodispersed system; however, the estimates of the average particle sizes that were obtained by various methods are different. According to ultrasonic acoustic spectral analysis, the average particle diameter is about 140 nm, while according to dynamic light scattering, it is approximately 350 nm. Electron microscopy of a xerogel obtained from a sample after the removal of water gives an estimate of the average particle diameter of about 250 nm [12]. This indicates that the region of the modified dielectric permittivity is larger than the region with changed density; thus the particle may be regarded as a dense core and a loose corona (or coat). The absence of the Cotton effect in Panavir solutions indicates the structurelessness of particles, i.e., the particle core, if it exists, does not have any appreciable orientation helicoidal ordering. At the same time, these particles are chiral, as indicated by the optical activity of the solution [6, 12, 15].

Since the viscosity of the Panavir solution at any concentration does not differ from that of water, i.e., the friction of particles is negligible, it should be assumed that the coronas of the particles do not overlap. Then, from the above data on the solubility we can estimate the average molecular weight of the particles, which is not less than 3×10^9 Da.

If the core is electroneutral as a whole, the negative charge may be distributed on the surface and in the corona of the particle. The distribution of the charge density, as well as the density of chain segments and

the coefficient of friction, must be correlated with each other and can be described by the function

$$f(r) = n_s(r)/\tilde{n}_s,$$

where $\tilde{n}_s$ is the equivalent density for the uniform distribution of segments in the particle corona.

Since it is not usually possible to establish the exact distribution of the segments, in the calculations it is assumed that the density of segments decreases nonlinearly, but monotonically from the core to the boundary of the particle. Perhaps it is the low density of segments at the boundary and the high hydration energy of sugars that leads to mutual steric repulsion of Panavir particles from each other. Analysis of the influence of the non-uniform density distribution of segments around the core showed that the amendment to the zeta potential at a given electrophoretic mobility is small and can be neglected at a reasonable accuracy of the measurements for Panavir particles [16, 17].

The surface potential, φ, of a particle is related to the zeta potential by the following equation:

$$\varphi = \zeta(1 + z/a)e^{\kappa z},$$

where ζ is the zeta potential, a is the particle radius, z is the distance from the particle surface to the sliding plane, and κ^{-1} is the Debye shielding length (it is less than 1 nm for the physiological conditions). Since the z value is impossible to determine directly, it is conventionally taken to be 0.5 nm [18].

If we assume that a particle is dielectric with the constant ε and the diameter r, and the potential on its surface is created by a point charge located in its center, the charge magnitude would be equal to $q = 4\pi\varepsilon_0\varepsilon r\varphi$, which gives a magnitude on the order of 20 elementary charges (e) for $r = 150$ nm and $\varepsilon = 80$. Any spherically symmetric distribution of the charge within the particle will match this average potential. The average charge density in the particle is about $6 \times 10^{-6}\ e$ nm^3. Note also that the resulting number of elementary charges is obviously much smaller than the number of functional groups (~10^6) in the Panavir particle.

The ability of Panavir particles to spread over the surface of a solution at a saturation concentration, as well as the substrate surface, indicates that they practically do not have mechanical elasticity [12, 15].

3. INTERACTION OF PANAVIR PARTICLES WITH CELLS

When Panavir particles enter the bloodstream, they are quickly carried by the bloodstream throughout the body. Given that the average velocity of the blood flow in the major arteries is about 0.5 m s^{-1}; in veins, about 0.15 m s^{-1}; and in the capillaries, up to 10^{-3} m s^{-1}, it can be assumed that after intravenous injection the interaction of Panavir particles with the blood and endothelium cells starts immediately and with all other cells starts in a time from several seconds to several minutes.

For interaction of the particles with the cell, it is necessary that their collision occurs. The frequency of collisions can be estimated from the model of the collision of solid particles with a sphere. Assuming that the particle diameter, d, is equal to 350 nm, the molecular weight is equal to 10^9 Da, and the density is equal to the density of water, we obtain that the particle volume $V = 2 \times 10^{-20}$ m^3 and the mass $m = 2 \times 10^{-17}$ kg. Then, from the Maxwell distribution it follows that the average value of the thermal velocity of the particle

$$\langle v\rangle = \sqrt{\frac{3kT}{m}} \approx 2.5 \times 10^{-2}\ \text{m s}^{-1}.$$

Taking the blood volume to be 4 L, it is possible to estimate the concentration of the particles immediately after infusion: $n = 10^{11}/(4 \times 10^{-3}) = 2.5 \times 10^{13}$ m^{-3}. The frequency of particle collisions with an element of the cell surface, ΔS, can be roughly estimated by assuming that in each microvolume one-sixth of all the particles move toward a cell. Then the collision frequency $\Delta z = \frac{1}{6} n\langle v\rangle \Delta S$, and after summing we obtain $z = \frac{2}{3}\pi R^2 n\langle v\rangle$; and, for example, for the cell of diameter of 15 μm the collision frequency will be approximately 74 s^{-1}.

After a collision, the interaction of Panavir particles with cells may be direct with some sites of the membrane, or indirect when binding to receptors.

For example, TLR4 may be a possible candidate receptor; it is expressed in monocytes and macrophages, as well as dendritic and endothelial cells. Receptors of this type (toll-like receptors) are most common in vertebrates and invertebrates, some of their units are found in bacteria and plants; an affinity for polysaccharides has been observed in them [19]. When ligands are bound to toll-like receptors, phagocytes are activated and secrete cytokines and other substances that cause the development of the inflammatory response. Since Panavir has an anti-inflammatory effect, this path seems to be unlikely.

A receptor path usually results in the internalization of a bound particle, with its subsequent destiny being determined by the type of a receptor, the particle size, and the cell type. For large particles such as Panavir particles, the main way to penetrate the cell is macropinocytosis [20–22]. If these pathways of particle penetration into the cell were effective, at a dosage of about 10^{11} particles one would expect manifestations of a toxic action of the drug. The absence of toxic action means that the probability of the Panavir particle penetration into cells is low.

An analgesic effect is usually associated with the influence of analgesic and anesthetic agents on sodium channels in the cell membranes of neurons, as well as the synthesis of prostaglandins, the activity of cyclooxygenases (COX1 and COX2), and secretion of serotonin, etc., although the molecular mechanisms for such impacts are not yet fully understood. One of the possible mechanisms of analgesic action is associated with changes in the lateral pressure profile in the cell membrane, which lead to conformational changes of ion channels and membrane-bound proteins [23–25]. Apparently, the latter mechanism can also occur in the case of Panavir, but the adsorption of Panavir particles on the membrane is necessary for its manifestation.

Since Panavir has an effect on such different cells types as nervous and immune cells, the mechanism of its action should be versatile and most likely it does not involve binding of the particles to specific receptors. The immune response to Panavir can be both direct and caused by its action on the central nervous system [11].

The cells of the immune system have dimensions of 10–30 μm, i.e., they are 1.5–2 orders of magnitude larger than Panavir particles. In this regard, the interaction of a Panavir particle with the cell membrane can be approximately regarded as an interaction of a soft nanoparticle with a semi-infinite space limited by a plane. Obviously, in order to impact cells without interacting with receptors, the Panavir particles should be reversibly or irreversibly adsorbed onto the cell membrane. It is convenient to divide the interaction of a particle with a membrane into two stages: before contact and after collision.

At both stages the interaction is determined by the van der Waals and electrostatic forces. Under physiological conditions, electrostatic forces are effectively shielded to a distance of less than 1 nm due to high ionic strength. At smaller distances, they may be both attractive and repulsive forces depending on the local properties of the cell membrane.

In mammalian cell membranes, 10–40% of the lipids are negatively charged, the remainder are neutral or zwitter-ionic lipids [26]; therefore, (without considering the charges of proteins) the cell membrane has an average negative charge. However, for particle interaction with the membrane, it is not the average value of the charge that is important, but rather the local one, which may be positive or zero. With the approach of a particle to a membrane site with a significant negative charge, a collision may or may not occur; but there are many more uncharged or positively charged sites, so with a greater probability the particle will collide with the membrane, even without considering the van der Waals interaction.

The van der Waals interaction of a particle with a membrane before contact has two constituents corresponding to the core and coat, which are separated from the membrane by a water layer. The energy of interaction depends in a complicated way on many parameters, such as particle sizes, the distance between a particle and a plane, and the "characteristic length" of an interaction; but for small distances z between a particle and a plane we can use the Gregory approximation:

$$V_{\mathrm{vdw}} = \frac{Aa}{6z\left(1 + 14\frac{z}{\lambda}\right)},$$

where A is the Hamaker constant, a is the particle radius, and λ is the "characteristic length" of an interaction, which is usually assumed equal to 100 nm.

The Hamaker constant can be expressed through the Hamaker constants for the pure phases of substances of particles, membranes, and water [27]. The Hamaker constant for water is approximately equal $A_{\mathrm{W}} = 4.4 \times 10^{-20}$ J; the value $A_1 = 1.5 \times 10^{-19}$ J is usually taken for hard surfaces; and for polymer coatings of nanoparticles, it is $A_2 = 7 \times 10^{-20}$ J. The Hamaker constant for the interaction of a soft particle with a plane surface then will be on the order of 10^{-21} J for a uniform and dense distribution of the polymer segments in a coat [17].

Calculation of the potential energy profile of interaction between a particle with a diameter of 100 nm and a surface at the charge density of 0.01 nm^{-3} and ionic strength of 10 mM indicates the presence of a barrier with a height of about $3kT$ at a distance of about 1 nm between a particle and a surface. Increasing the ionic strength, particle size, and a decrease of the charge density will result in a decrease in the barrier height. Thus, about 5% of the particles will overcome the barrier and collide with the cell membrane. At distances of less than 1 nm, the van der Waals forces are superior to the electrostatic ones and result in the adhesion of a particle to the membrane.

Further, there may be different scenarios depending on the distribution density of segments in a coat. At high density, even a small area of interaction provides the formation of a large number of van der Waals contacts and the binding of particles to the membrane becomes irreversible. At a low density of segments, the binding of an elastic particle is reversible; in the case of Panavir, particles do not have elasticity, so it is necessary to consider the possible spreading of a particle on the cell surface.

Since the cell membrane is a dynamic structure, the spreading cannot be imagined mechanistically: the particle surface and the cell membrane adapt to each other so that the energy of the particle on the membrane is at a minimum. With the spreading of a particle, the contact area between it and the membrane increases many times, as well as the van der Waals interaction energy. Thus, for example, at the spreading to a thickness of 10 nm (in the absence of a core), a

particle forms a disk with a diameter of about 200 nm on the cell surface. In the presence of a core, the disk sizes decrease, but still remain "macroscopic."

Calculations using DLVO theory show that in the interaction of particles with a roughened surface the irreversible binding corresponding to the first energy minimum increases significantly with increasing ionic strength [28]. The cells may have a roughened surface due to caveolae and other irregularities of the membrane that are characteristic of many cells, especially macrophages, neutrophils, and dendritic cells. In the presence of divalent ions, the value of the free-energy maximum can be significantly reduced due to the formation of ion bridges [29].

The interaction of Panavir particles with the cell membrane can lead to the formation of rafts, which are small lipid domains of a transitional character. The formation of domains is practically insensitive to the details of molecular interactions that lead to the immobilization of a particle on the membrane [30]. At the same time, the formation of rafts results in significant changes in the lateral pressure profile, which is required for the analgesic action of Panavir.

In the interaction of nanoparticles with the membrane, the local changes of surface curvature near membrane-bound proteins and their complexes, as well as its thermal fluctuations, due to which the local electric fields can vary greatly via the flexoelectric effect, may play an important role [31].

However, any significant change in the properties of the membranes of many cells (e.g., due to the adsorption of Panavir particles) may lead to toxic effects [23–25]; therefore, this mechanism can occur only in a small number of cells, or for a short time.

Considering the extremely low toxicity of Panavir and the significant frequency of collision of its particles with cells, it can be assumed that this effect is due to the mechanical activation of sensitive cells. Such activation is known for cells of the immune system; it is mediated by cytoskeletal reorganization [32].

Let us take as a rough model the idea that in a collision a particle hits the cell membrane by a "thorn" of the polysaccharide chain. The pressure, P, that is exerted on the membrane can then be estimated by equating the kinetic energy and the work of compression of the membrane. Then, $P = 3kT/(2lS)$, where S is the area of contact with membrane (no more than 0.5 nm^2), l is the length of a "thorn" (a Kuhn segment length of about 5 nm). After substituting the numerical values we obtain a pressure $P > 100$ atm. Evaluation of the pressure created by a small thermally activated particle is also valid for soft nanoparticles with a sufficiently large diameter. A soft particle can be approximately regarded as a system of weakly bound molecular fragments or moving point particles with the thermal energy $\sim kT$. Such collisions with the cell can result in significant deformation of the membrane and mechanical impact on the cytoskeleton, which would be enough to activate, for example, macrophages.

Most likely it is the absence of mechanical elasticity that determines non-destructive mechanical impacts on the cytoskeleton and distinguishes Panavir particles from other biologically active particles that have a similar size and charge, but are characterized by a significantly higher toxicity.

This model of activation of immune cells is consistent with the versatility of the antiviral action of Panavir and its extremely low toxicity.

Assuming that the cytoskeleton of the immune system cells serves as a phenomenological target for Panavir, one can also explain the efficiency of the Panavir action in preventive schemes against the rabies virus [33], bacterial infection, and as an antidote.

CONCLUSIONS

Thus, a simple physicochemical model of the interaction of Panavir with the cell makes it possible to qualitatively explain its antiviral, anti-inflammatory, and analgesic effects, as well as its low toxicity. The effects of Panavir are most likely to be due to a direct collision action on the cytoskeleton of sensitive cells, particularly immune cells. The prophylactic action of Panavir can also be explained in terms of these representations. The main conclusion of this work is that all these effects of Panavir can manifest themselves without binding of its particles to any supramolecular chiral receptor; a key feature of panavir particles is their absence of elasticity.

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