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Effects of Physicochemical Forms of Phenazepam and Panavir on Their Action at Ultra-Low Doses

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A concept of physicochemical forms of biologically active substances introduced in investigation of the action mechanism of ultra-low doses allows qualitative explanation of the main effects of ultra-low doses, chemical diversity of biologically active substances, and physical boundaries for these effects. Phenazepam was shown to possess activity in ultra-low doses only in disperse state, in the form of nanoparticles with a diameter <100-300 nm; these nanoparticles appear as micelles of surface active substances and solvated. Panavir possesses pharmacological activity in ultra-low doses and appears as nanoparticles with a diameter of 200-300 nm, which have uncompensated negative surface charge and polymer nature.

Key Words: *pharmacological activity; ultra-low doses; nanoparticles; phenazepam; panavir*

We previously showed different effects of phenazepam (PZ) in ultra-low doses (ULD) [4] depending on its physicochemical form (molecular solution or molecules condensed into nanoparticles) and hypothesized the formation of local PZ concentrations comparable with the therapeutic ones at the scale of cell and larger (L) when 250 nm PZ nanoparticles penetrate cell population [8]. It resembles the action of "hot particles", when local irradiation doses are high, while mean incorporated dose is insignificant [10]. This phenomenological model proposed by Timofeyev–Resovsky includes multiscale target discretization and is used for various biological objects in microdosimetry [3].

This study provides more detailed discussion of the issues of physicochemical forms of drugs at ULD, although authors adhere to the theses formulated previously [8]. Effects of ULD of PZ and immunomodulator Panavir were experimentally studied [7,9], their physicochemical forms possessing pharmacological activity in ULD were determined, peculiarities of the molecular mechanisms of action are discussed.

MATERIALS AND METHODS

For evaluation of the role of physicochemical (molecular and aggregate) PZ form in ULD, we studied biological activity of PZ water solution (pseudomolecular) prepared by consecutive dilution in concentrations of 10^{-3}-10^{-20} M, and PZ solution obtained using method [4] and also using serial dilution at the same concentrations. The solutions of the test drugs were prepared using bidistilled water under conditions of clean GMP zone of NIK pharmaceutical company.

Pharmacodynamics of PZ in ULD was studied under conditions of single and multiple dose administration in standard pharmacological tests and in comparative investigation of interoceptive differentiation

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properties using PZ in usual pharmacological and ULD doses in operant model of medication differentiation in rats. Disperse components of the solutions were assesses using dynamic light scattering approach.

Panavir substance (manufactured by NIK) was also used in the experiments. Its biological activity was evaluated using the model of artificial peritonitis induced in rats by intrapertoneal administration of aqueous solution of autologous faeces (≈3 μg per rat).

RESULTS

Experimentally observed minimal concentrations in ULD effects were limited by saturated vapor pressure of the corresponding bioactive compounds (BAC), what takes of the table the issue concerning reliability of ULD effects at concentrations less than 10^{-23} M and therefore the issue concerning energetic and information interactions in pharmacology and homeopathy.

PZ solubility in water experimentally determined using multifield microscopic visualization at 36.6°C was $<10^{-7}$ M. There were no statistically significant biological shifts after intraperitoneal administration of various solution concentrations (10^{-8}-10^{-20} M), prepared using the above method to experimental animals (10 rats per each concentration).

At the same time, reliable maximal biological activity for the solution prepared with Tween-80, where micelle formation is imposed by surface-active substances (SAS), was noted at a concentration of 10^{-12} M [4]. The observed pharmacological action of PZ at ULD differed from that for PZ at normal concentrations. Thus, PZ demonstrated pronounced anxiolytic effect in conflict situation without inducing locomotion coordination disturbances, sedation, and myorelaxation typical of conventional doses. There also was no withdraval effect after discontinuation of long-term PZ administration at ULD. Differences of PZ effects in normal dose range and in ULD, as assessed in the drug differentiation model, suggest that PZ effect at ULD is associated with the target different from that when PZ acts in normal dose range. We believe that PZ action at ULD is effectively associated with collective states spatially distributed at the scale of L receptor targets, that are essentially different from those activated by normal PZ doses, what is apparently reflected in low toxicity of PZ ULD. Micelle size at the concentrations of 10^{-4}-10^{-6} M appeared to be 100-300 nm as measured by the dynamic light scattering. Method sensitivity was insufficient to carry out the measurements at lower concentrations. Thus, simple PZ dilution in ULD was experimentally established to result in no significant biological shifts. At the same time, addition of SAS Tween-80 to the solution results in aggregation of PZ molecules into micelles and simulates ULD effects. Bearing in mind that PZ nanoparticle of that size contains ~10^9-10^{10} molecules, one can easily estimate that characteristic area where the concentrations are close to the pharmacological values exceeds the size of the cell. The concentration of exogenous compounds under homeostatic conditions is substantially lower than ULD concentrations, whereas chemical diversity in the conditions of contemporary ecology accounts at least 10^4 compounds, for which ULD effects were observed [2]. It means that in addition to the size and chemical composition of nanoparticle, the pharmacological active mass of the drug (BAC) in it is also important. For PZ, the mass of such nanoparticle is 10^{-14}-10^{-13} g.

Then we discuss ULD effects observed in Panavir solutions with 200-300 nm nanoparticles characterized by uncompensated negative surface charge and formed by strongly bound associates of plant polysaccharides belonging to hexose glycosides with known composition [8].

The lack of nanoparticle aggregation can be explained by solvate cover and weak Coulomb repulsion. Experimentally established weight of Panavir nanoparticles is 10^8-10^9 Da or 10^{-16} g [8]. The dose–effect relationship has a bimodal pattern at the ULD area (Fig. 1). In addition, maximal efficiency in the model of peritonitis at the range of normal concentrations corresponds to optimal Panavir concentration in equivalent to therapeutic dose of 200 μg, which confirms the correctness of experimental model choice. Thus, following peculiarities of drugs physicochemical forms, acting at ULD, were noted in both reviewed examples: compactization into the 100-300 nm nanoparticles; presence of a factor preventing aggregation, *e.g.* surface charge or SAS solvation; critical amount of BAC.

These results are also in line with the data for low-concentration solutions of BAC compounds [5,6]. Typical size of Panavir nanoparticles provides high bioavailability, including penetration through the blood-brain barrier.

Some peculiarities of ULD BAC action have no clear explanation [1].

This first of all concerns minimum (or absence) of BAC activity with increasing concentration above the maximum ULD. Within our approach this can be explained by enlargement of BAC particles up to microscopic sizes, which prevents efficient BAC transport into the intercellular space, and consequently dramatically reduces bioavailability. With further increasing BAC concentration, the transport processes become significant, which was associated with efficiency and toxicity of BAC in normal range of pharmacological concentrations. Provided qualitative discussion also explains multimodality of the dose–effect relationship with polyvalent drug activity.

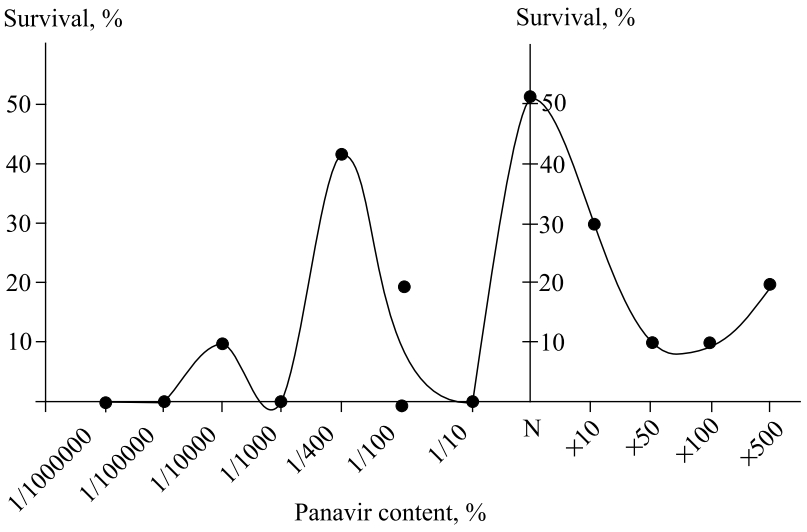

Fig. 1. Bimodal survival curve for animals with experimental peritonitis. N: therapeutic dose equivalent in conversion to one experimental animal. ×10-×500: factor by which the therapeutic dose was exceeded.

In addition, different physiological effects of ULD and pharmacological concentrations can be associated with different targets for molecularly dissolved drug and its nanoparticles. This manifests in different rate of drug delivery to the target, bioavailability, membrane permeability, and intracellular reactions for different physicochemical forms.

Biophysical criteria for ULD phenomenon also become evident within this framework. The extent of local transfer of the drug molecules after drug nanoparticle penetration into the cell population (organ) has to correspond to the size of the target, whereas drug concentration in this area has to correspond to its number of hits [3]. In addition, all periods of local concentration decay down to the critical values have to be longer that typical time of target bioregulation. The criterion formulated above is not unique. Generally it's the time inside cell and it's substantially shorter than time of diffusion for drug molecules, and area of high local (pharmacological) concentration includes macroscopic number of cell higher than 10^1, what facilitates fulfillment of condition of multiple hits. Thereby, apparently the ULD phenomenon was noted for a huge chemical diversity – more than 10^4 BACs [1,2].

When the number of drug nanoparticles that hit the target is comparable with the dispersion of this value, then the variability or irreproducibility of ULD results are observed [1,2]. Taking into account results of physicochemical investigations [5,6,8], the ULD results [1,2] we generally obtained using pseudomolecular solutions with unclear (uncontrolled) and possibly strong aggregation factor. For example, in micelle formation, amphiphilic components of the drug could play the role of SAS. Chiraliry, as the supramolecular parameter, also may result in aggregation of the compound at the low concentrations. Physicochemical scenarios for compound nano-condensation may be different. That property is fundamentally coupled with the parameters of spectrum of its electron states, characterized by quantum-mechanical nanosize condition:

$$E_n \approx kT,$$

where E_n – distance between electron levels, T – temperature, k — Boltzmann constant [11]. Such single transfers may be activated relatively easily for the formation of long-living connection cluster and with subsequent compound aggregation.

Thus, our study showed that ULD concentration range is limited physically by saturated vapor pressure. PZ possesses pharmacological activity at ULD only in disperse state in the form of 100-300 nm nanoparticles; in addition, nanoparticles appear as SAS micelles and solvated. Panavir exhibits biological activity at ULD and appear as 200-300 nm nanoparticles with uncompensated surface charge and polymer nature. The study of ULD action mechanism resulted in introduction of the concept of physicochemical drug (BAC) forms, that allows qualitative explanation of main properties of ULD effects within the framework of available phenomenological models of biological activity. Possible reason for vast chemical variety of BACs, for which ULD effect was noted, has been revealed.

In conclusion it should be noted that despite qualitative review of the action of drug (BAC) physicochemical form actions, our approach, apparently, will allow to make the selection of drugs and their pharmaceutical forms for ULD scientifically grounded.

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