Regulatory Activity of Ultralow Doses

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For many years biological properties of ultralow doses (ULD) of medicinal preparations are studied with experimental and clinical methods under the aegis of the "Materia Medica Holding" Research-and-Production Company. It was shown that medicinal preparations in ULD possess specific activity. Their specific effects and previously unknown phenomena were evaluated.

These data allowed us to introduce a new class of medicinal preparations, ULD of antibodies, into medical practice. In addition to this, achievements in various fields of theoretical and practical medicine (immunology, physiology, and homeopathy) were summarized. Our results and published data allowed us to reconsider historical experience of homeopathy, which uses ULD for more than 200 years.

Of considerable interest is the method for preparing extremely diluted solutions developed by the founder of homeopathy S. Hahnemann – a combination of multiple dilutions of the initial substance with exposure to exogenous factors. Published data show that ultrasound and electromagnetic field can serve as exogenous factors, while Hahnemann used mechanical shaking only. This technology was designated potentiation and allows obtaining the so-called potentiated solutions. It is obvious that this empirical method of potentiation renders medicinal preparations in ULD biological active. Physical mechanisms of potentiation remain unclear. It should be emphasized that potentiated solutions exhibit high biological activity not only in molar but also in submolar (beyond Avogadro's number) concentrations.

As a field of medicine, homeopathy is close to clinical pharmacology. Homeopathy suggests detailed clinical investigations of the effects produced by medicinal preparations. Clinical observations are performed on healthy volunteers.

The fundamental methodological principle of homeopathy is individualization of the prescribed medicinal preparation (principle of similarity). It was empirically established that the sensitivity to ULD of arsenic is typical of individuals with certain constitutional characteristics (lean, with thin bones, light skin, "geographic" tongue, etc.), behavioral features (convenience in warm places, consumption of considerable amounts of water, and pedantry), and predisposition to chronic diseases.

We believe that the individualized use of medicinal preparations in ULD produces hyperergic reactions, probably mediated by immune mechanisms of hypersensitivity. The existence of morphological and functional characteristics, a systemic marker of individual sensitivity, is the second most important parameter (after technology) for the rational analysis of homeopathy.

Strangely enough, Hahnemann initially used preparations in "material" doses. Then he decreased the concentration of substances due to the necessity of reducing the degree of hyperergic reactions. Ultralow concentrations of potentiated solutions are a result of potentiation process. The effects of potentiated medicinal preparations are determined by technology (number of dilutions), but not the concentration of substances in the potentiated solution. The number of dilutions is not opposite to the substance concentration.

Studies were performed with the centesimal scale of dilutions. Dilutions of C30, C200 and C1000 correspond to equivalent concentrations of 10^{-60}, 10^{-400}, and 10^{-200} wt %, respectively. The main pharmacopoeial advantage of potentiation is standardization of ULD for various forms of medicinal preparations (*e.g.*, tablets, ampoules, and granules).

Experiments performed in the last 15 years demonstrated high biological activity of substances in ULD [10-12,49,51]. The use of standard test systems at various research institutions confirmed reproducibility of the effects produced by ULD.

The principal conclusion drawn from our studies and published data is the same tropism of the substance and its ULD to substrate or physiological process. Experiments on isolated neurons showed that substances in physiological (therapeutic) doses and ULD produce qualitatively similar effects (membrane depolarization) but these effects differ quantitatively [44].

From the pharmacological point of view the most attractive property of ULD is their ability to modify the effect of therapeutic doses of the same agent during combined treatment. This method for administration of medicinal preparations was named bipathic technique [42].

The phenomenon of bipathy was discovered in 1996. Prednisolone in toxic doses and potentiated prednisolone (C30) were given to experimental animals

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intragastrically and perorally, respectively. Control animals received prednisolone alone. It was found that potentiated preparation prevented the development of destructive changes in the liver, adrenal glands, lymph nodes, and gastric mucosa observed after long-term treatment with prednisolone. Potentiated prednisolone normalized white blood count. The contents of blood ATP and alkaline phosphatase activity in neutrophils remained unchanged. Lactate dehydrogenase activity in neutrophils and ATPase activity in lymphocytes were not suppressed.

Further studies demonstrated the fundamental physicochemical character of the bipathy phenomenon:

- potentiated ATP added to ATP solution reduces the rate of its hydrolysis;
- potentiated mercury nitrate added to mercury nitrate solution increases activity of mercury ions in the standard electrochemical system;
- potentiated antibodies change the binding constant in the antigen-antibody reaction (was demonstrated for a number of peptides).

For investigation of the phenomenon of bipathy, M. B. Shtark proposed a method of induction of long-term posttetanic potentiation (LTPTP) in hippocampal slices. Antibodies to S100 protein (AB-S100) were used as the pharmacological agent. AB-S100 blocked LTPTP in hippocampal slices. Potentiated AB-S100 abolished the inhibitory effect of AB-S100 in physiological concentrations.

Bearing in mind previous data about the phenomenon of bipathy we concluded that elimination of LTPTP blockade is determined by modifying properties of potentiated antibodies [43].

Further studies confirmed the assumption that activity of antibodies in ULD is related to the phenomenon of bipathy.

Patients with chronic alcoholism and drug abuse hospitalized at the Center for Mental Health (Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences) in 2002 received ULD of AB-S100 and antibodies to morphine, respectively, for 2 months. The decreased concentration of natural antibodies in the plasma returned to normal, which correlated with the improvement of mental state. This effect was characterized by high specificity: ULD antibodies to morphine had no effect on AB-S100 concentration and vice versa; synthetic psychotropic agents did not change the concentration of natural antibodies. Our experiments with ULD of AB-S100 showed that their modifying effect is realized at various levels of organization of neuronal structures *in vitro* and *in vivo* (giant isolated neurons, organotypic culture of the nerve tissue, brain slices, and whole organism).

In recent years the concept of natural antibodies was revised. It was shown that natural antibodies are involved in the multifactor regulation of natural functions [7]. Activity of these antibodies does not correlate with autoimmune disorders. The repertoire of natural antibodies reflects the molecular specificity of each adult organism [50]. Natural antibodies to various low- and high-molecular-weight peptides, surface membrane structures, and nuclear components were revealed. It should be emphasized that 50% of circulating natural antibodies are presented by IgG.

Autoantibodies against various endogenous peptide regulators are involved in their transport to membrane receptors preventing them from proteolysis. Published data show that natural antibodies possess catalytic (enzyme-like and abzyme) activity. These antibodies affect cell proliferation, myelinization, and activation of membrane ion channels. There are data that natural antibodies are transported through membrane structures and blood-tissue barriers. Complex quantitative analysis of autoantibodies to endogenous regulators is used for the diagnostics of diseases [30]. Previous studies revealed that anti-idiotypic antibodies against protein S100 modify the biological effects of this antigen [29]. Taking into account modern notions on the role of natural antibodies, we hypothesize that modification of functional activity of certain autoantibodies with ULD of the corresponding antibodies allows "regulation of the regulator"

The concept of immunochemical functional system of homeostasis postulates that metabolic and immunological processes in the organism are closely interrelated [20]. It can be anticipated that ULD of antibodies to endogenous regulators can modify their activity. Experiments showed that ULD of antibodies to γ-interferon (G-IFN) 3-8 fold intensify its production by lymphocytes [59,60].

Autoantibodies to endogenous regulators in ULD modulate functional and metabolic processes associated with these regulators. For instance, potentiated antibodies to G-IFN modulate synthesis of G-IFN, interleukin-4, and interleukin-8 in lymphocytes.

The directionality of changes produced by ULD of potentiated antibodies depends on the initial functional state. For instance, ULD of antibodies to erythropoietin and granulocyte colony-stimulating factor increase erythropoietic and granulocyte/macrophage colony-stimulating activity during cytostatic myelosuppression, respectively, and produce an inhibitory effect during immobilization stress.

Combined treatment with ULD and physiological concentrations of AB-S100 suppressed generation of action potentials in neurons with high spontaneous activity, but stimulated this process in neurons with low spontaneous activity.

For understanding of the mechanisms of changes produced by potentiated antibodies, it is important that

AB-S100 in ULD produce a membranotropic effect, modulate synaptic effectiveness, and change the content of intracellular transmitters. Antibodies to NO synthase activate this enzyme, stimulate NO production, and increase intracellular cGMP content.

Activity of ULD of antibodies to some endogenous regulators S100, histamine, granulocyte colony-stimulating factor, erythropoietin, tumor necrosis factor-alpha (TNF-α), G-IFN, endothelial NO synthase, and prostate-specific antigen (PSA) and exogenous compound morphine was experimentally studied. High therapeutic effectiveness of most preparations was confirmed in clinical studies.

Experiments with AB-S100 showed that potentiated antibodies produce a variety of effects:

shift of electrical characteristics of calcium channels byalong the voltage axis, reduction of the maximum amplitude of calcium current, and partial inhibition of Ca^{2+}-dependnet potassium current;

voltage-dependent selective effect on synaptic effectiveness;

blockade of the inhibitory effect produced by physiological doses of AB-S100 on LTPTP;

universal sensitizing effect of Proproten-100 on the neuronal membrane. This effect was studied using potentiated forms of membrane modifier of ion channels cyclosporine and theophylline modulating metabolism of intracellular messengers. The modifying effect of ULD was observed in this case. Preincubation of hippocampal slices and organotypic cultures in potentiated solutions of physiologically active substances producing strictly determined local effect was followed by changes in surface characteristics of neurons. Patch-clamp experiments showed that this effect is calcium-dependent: the observed changes were associated with modification of Ca^{2+} channels;

modulating effect on the monoamine system in various brain structures;

influence on the GABAergic system;

modulation of pulse activity in neurons of the hippocampus and hypothalamus;

normalization of the rate of self-stimulation in the lateral hypothalamus;

anxiolytic and antidepressant effects of ULD of AB-S100 comparable with the influence of diazepam and amitriptyline, respectively. It should be emphasized that ULD of AB-S100 exhibit no sedative and myorelaxing activity;

antiischemic and antihypoxic effects surpassing the effects of piracetam, cavinton, and mexidol, respectively.

The results obtained in experiments with ULD gave rise to new therapeutic methods and on the other hand raised many general biological and scientific questions. We shall dwell on several problems.

As was mentioned above, substances in ULD produce three types of effects:

specific molecular and cellular effects qualitatively similar to the influence of substances in standard doses, but less pronounced;

hyperergic reactions during individualized treatment (homeopathic);

modifying effect (phenomenon of bipathy and antibody-mediated variant).

Specificity of the effect produced by ULD indicates that their physicochemical properties remain unchanged in potentiated solution (submolar concentrations). Probably, the modified structure of solvents serves as a carrier of information about the initial substance, i.e. new relationships are formed between molecular, atomic, and dualistic (corpuscle-wave) particles of the solvent. The nature of these relationships is beyond the resolution capacity of modern physical methods. Fine chemical mechanisms underlying *in vivo* and *in vitro* effects of substances in ULD remain unknown. The effects of ULD should be studied with conclusive experimental and clinical methods. The systemic approach is required for understanding of these changes.

During potentiation the solvent retains the information about various substances, and it can be hypothesized that specific properties of the substance manifest in the structure of newly formed bonds (relationships), but not in their nature. The potentiated solution is structuralized in accordance with fine structure of the initial substance. Since the solvent is a complex dynamic system, the structure of the potentiated solutions is dynamic in nature, i.e. represent a constellation of solvent particles, a complex spatial formation. The structure of this spatial formation reflects its semantic content.

The empirically derived method of potentiation open the way to the world of semantically organized constellations, which serves as an example of spatial organization at the semantic level. We believe that semantic organization is typical of living and nonliving nature. Semantically organized constellations are the apparatus for semantic analysis that involves philosophical and biological principles (reflection and memory, respectively). The principle of semantic organization is evolutionarily determined. Probably, biological evolution was preceded by semantic evolution. The general and simple principle of this evolution is that each semantic (material) factor developed during evolution should produce a system of hierarchical relationships with semantically simple factors. We live in a system of developed semantic relationships. That is why any exogenous factor (including pharmaceuti-

cals) causes the evolutionarily determined response. The unique systemic response does not depend on specific molecular, cellular, and functional reactions. It is determined by a unique combination of these reactions reflecting semantic characteristics of the exogenous factor (pharmaceuticals and other agents).

Cascade metabolic reactions mediated by secondary messengers, calcium, adenylate cyclase, phosphoinositol cycle, and protein kinases are the most prominent example of constellations. Each molecule can be simultaneously involved in various constellation relationships. Integration of constellations into complex spatial formations (semantic synthesis) is realized via various mechanisms similar to interference. Homeostasis and structuralized vital activity of the organism are based on functioning of numerous constellations. The systemic analysis of behavior performed in the theory of functional systems (P. K. Anokhin and K. V. Sudakov) is of great interest in this respect. Probably, common properties of functional systems can be extended to constellation relationships.

In our opinion the phenomenon of bipathy is very close to the particular feature of functional systems, advanced programming of favorable adaptive results. The constellation combines not only molecules of various classes, but also their atomic and subatomic particles and wave characteristics. It is reasonable that reconstructions accompanying semantic analysis at fine and less inert levels of constellations proceed more rapidly than at the molecular level. During combination treatment with the preparation in standard concentrations and ULD, these ULD organize constellation relationships in advance and reduce the intensity of metabolic recognition. Probably, the holographic principle of organization in functional systems proposed by K. V. Sudakov extends to semantic constellations. By analogy with the dual structure of holograms (reference and object waves), it can be suggested that semantic constellations are characterized by dual organization. This species and individual organization was previously designated as horizontal and vertical organization, respectively.

Pharmacologic and immunologic assay showed that any substance produces the species-specific response. Therefore, molecules and submolecular particle in constellations formed during the response are characterized by species-specific spatial relationships. The structure of constellations reflects semantic architectonics of the active factor. It should be emphasized that in one of holographic planes in the constellation, the individual type of spatial relationships between its components remains unchanged.

Studies of substances in ULD showed that they initiate molecular and cellular reactions "undetectable" at the clinical level. During the individualized use (with phenotypic markers) the molecular and cellular response undergoes transformation into the systemic reaction, which is similar to intoxication with the substance.

We believe that the cause of this phenomenon is the following. When semantic architectonics of the active factor coincides with the structure of individual relationships in the responding organism, the individual (vertical) plane of constellations does not undergo transformation. Holographic reflection of the exogenous stimulus within the limits of molecular and cellular reactions is impossible. Therefore, the need for large-scale constellations arises (systemic hyperergic response).

Another postulate of holography is that properties of the whole are manifested in each elementary unit of hologram. This postulate extended to semantically organized constellations indicates that their structure should be reflected in each constituting molecule or submolecular particle. Since each molecule consists of heterogeneous determinates, all its regions should be spatially interrelated and correspond to dynamic reconstructions that constantly proceed in the constellation. These data indicate that the molecule is characterized by permanent, important, fine, and conformational transformations. Clearly the more complex is the spatial organization of endogenous molecules, the greater is their semantic and regulatory role. Therefore, polypeptides and proteins with complex tertiary and quaternary structures act as genetic regulators in the Nature. Similarly to potentiation, endogenous proteins and polypeptides undergoing continual conformational changes structuralize and organize constellation processes in the surrounding locus. These changes correspond to species and individual characteristics encoded in their spatial structure. The more severe is the constellation-and-semantic situation, the more spatially complex genomic product (or products) is required for the regulation (primary genetic control).

The degree of multicellularity increases during evolution, which is accompanied by an increase in the probability of stochastic events. A demand arises for the secondary genetic control over spatial organization of endogenous peptide and polypeptide regulators. The principle of holography is appropriate for characterizing this process. The immune system regulates spatial organization of the whole molecule by controlling its semantic region (epitope).

Probably, the major role of the immune system is control over spatial organization of functional activity in the organism. Due to low affinity preexisting immunoglobulins and T cell receptors undergo reversible binding to heterogeneous, but semantically similar epitopes, and regulate their fine hierarchical structure. Constellations of natural antibodies (idiotypic-anti-

idiotypic network, N. K. Jerne [22]) possess greater plastic capacities for the regulation. First, constant synthesis of anti-idiotypes provides the existence and independence of this network on immunoglobulin half-life. And second, constellations of antibodies contain information about semantic relationships and functional activity of antigens that should be involved in these relationships. Therefore, natural antibodies regulate physiological processes associated with antigens and maintain the hierarchical structure of vital activity (homeostasis).

These data indicate that the therapy with ULD has a number of positive aspects.

First, this therapy is sparing. Constellations, but not substances (medicinal preparations) are introduced into the body. This reduces the stages of primary metabolic recognition and integration of substances into primary constellation relationships. Quick effects of medicinal preparations in ULD were demonstrated in experimental and clinical studies. ULD of drugs, including morphine and ethanol, do not induce tolerance and dependence. Clinical trials revealed no complications after treatment with substances in ULD.

Second, by the efficiency of ULD of substances are comparable or surpass modern synthetic preparations (even in patients with severe and complex pathologies).

And third, substances in ULD (*e.g.*, antibodies to G-IFN) hold much promise for the prevention of diseases.

Neurobiological screening of ULD of antigens and the corresponding antibodies (morphine and antibodies to morphine; protein S100 and AB-S100) showed higher effectiveness of ULD of antibodies.

Potentiation of antibodies is the most promising variant of application of ULD, because it allows modification of fine regulatory system of natural antibodies. The data offer considerable scope for the therapy of pathological processes of different nature and localization.

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