Translational research into vascular wall function: regulatory effects of systemic and specific factors

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Abstract

Development of biomedical techniques has intensified the investigation of endothelial glycocalyx as an individual research object. The effect of vascular wall hydration on progression of pathological lesions, including atherosclerosis, has been revealed and examined. There is evidence that atherosclerosis is associated with water-sulfate and water-sodium exchange. The concept that atherosclerosis is initiated by deficiency of sulfur-containing compounds has been regarded. Glycocalyx protective function against damaging effect of oxidative stress on vascular wall is associated with accumulation and retention of antioxidants on luminal surface of blood vessel. The review deals with protective activity of antioxidant enzyme derivatives, computational methods to determine the principles of the glycocalyx functioning and modeling of the glycocalyx interaction with systemic and specific factors. A limiting influence of crosswind move from clinical practice to life science and contrariwise from life science to clinical practice on the development of translational medicine is emphasized.

Abbreviations: BTH: bovine testicular hyaluronidase; CAT: catalase; exSOD: extracellular superoxide dismutase; GAG: glycosaminoglycan; ROS: reactive oxygen species; SOD: superoxide dismutase; SOD-CHSCAT: superoxide dismutase-chondroitin sulfate-catalase conjugate

Introduction

The development of translational medicine increases the effectiveness of therapy and promotes implementation of new pharmacological agents into clinical practice. It should be noted that current achievements of translational medicine do not comply with expectations. This is true for cardiology. Meta-analysis of myocardial infarction studies in a large animal model revealed the following limiting factors hampering successful translation of experimental data to clinical practice: the choice of clinically relevant model and study design [1]. The time of outcome assessment, sex of the animals, and blinding of the operator significantly influence the results obtained and partially account for failures in transition to clinical application. Greater adequacy (anatomically, hemo- and pharmacodynamically, applicability of therapeutic regimens) of large animal models (compared with small animal models) to human body does not abolish the differences. Modeling of accompanying diseases and conditions, such as diabetes mellitus, hypertension, smoking, overweight, etc., in large animals complicates experimental study and considerably increases its cost.

The problem is aggravated by the variety of cardiologic disorders. Thus, therapy of acute conditions, e.g., thromboses, requires elimination of occlusion, an established vascular disorder [2]. This is achieved with plasminogen activators employed in clinical practice as thrombolytic agents [3]. It is noteworthy that plasminogen activator derivatives with a greater molecular weight compared with that of parent substances prevail in the majority of recent laboratory studies. Thrombolytics (Metalyse, Reteplace) currently used in clinical practice have smaller of similar molecular size compared with parent compounds. This discrepancy between clinical data and large body of experimental evidence poses a question about the productive direction of biomedical research, i.e., whether molecular size of plasminogen activator derivatives should be increased or decreased [3]. The validity of the chosen stage/type of acute cardiologic lesion for the success of translational medicine is another aspect for consideration.

Current biopharmacological research is aimed at the development of breakthrough therapies [4] and competition in the most promising areas [5], suggesting that the results of translational medicine are effectively used in clinical practice [6].

Setting of a research goal in the direction from clinical practice to life science (Figure 1 A) now predominates in the organization of biomedical studies. This approach is based on various levels of evidence and programs for drug development in clinical medicine and medical research [6]. Simultaneously, transition from life science to medicine can also initiate development of novel drugs (Figure 1 B). Productivity of this approach is strongly determined by the choice of research goal and direction parallel to the development of effective technologies for drug production. Combination of these approaches is successful, judging from the use of thrombolytics based on tissue and urokinase type plasminogen activators as therapeutic agents and development of recombinant DNA technologies for their production [2,3]. Obviously, harmonic balance between the directions from clinical practice to life science (Figure 1 A) and from life science to therapy (Figure 1 B), their crosswind move enhancing the effectiveness of translational medicine (Figure 1 C).

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Primary barrier for preservation of the vascular wall integrity

Cellular glycocalyx is a functionally significant component in the circulatory system [7,8]. Interest in the investigation of glycocalyx has increased with improvements of laser scanning microscopy techniques which allow reconstruction of visualized objects and their accurate analysis. Glycocalyx with a thickness of 2-3 µm was visualized on the surface of cultured endotheliocytes by the method of confocal scanning microscopy [9]. In murine carotid arteries glycocalyx (thickness 3.5–5.5 µm) was visualized by two-photon microscopy [10]. Glycocalyx is represented by an outer layer of macromolecules, predominantly protein-carbohydrate and carbohydrate-lipid complexes bound to the plasma membrane and together with blood plasma proteins forming a lining with specific structure and a vast range of functions [7,8,11] (Figure 2). Noninvasive diagnostic methods have been tested to analyze the relationship between the state of endothelial glycocalyx and cardiovascular risk in humans. The analysis employs data obtained with the use of orthogonal polarization spectroscopy (OPS) [12] and sidestream dark field imaging (SDF) [13]. A reverse relationship between the thickness of endothelial glycocalyx in the microcirculatory network and the risk of cardiovascular lesions has been established. The collected body of evidence indicates that endothelial glycocalyx is a potential barrier between health and vascular disease [15,16].

Glycocalyx destruction is an initial event in the vascular lesion development caused by pathological factors. It is an essential initial stage in diabetes mellitus-related microangiopathies and chronic venous diseases [17]. Ischemia/reperfusion, infection, diabetes and renal failure induce glycocalyx destruction [18-20]. A decrease in its volume occurring in hyperglycemia increases vulnerability of blood vessels and is associated with endothelial dysfunction (elevation of plasma hyaluronan) and coagulation activation in vivo (high blood contents of 1+2 prothrombin fragment and D-dimer of fibrin) [21]. Various pathological factors (hypercholesterolemia, inflammation, hyperglycemia, excess of salts in the body, shear stress, nephrotic syndrome, etc) destroy glycocalyx [22-24]. It was suggested that glycocalyx destruction detected as changes in its composition and a decrease in its thickness has an important role in the development of endothelial dysfunction [22,25] and develops in patients with acute coronary syndrome [26]. The diversity of pathological factors inducing glycocalyx destruction that leads to progression of various
diseases implies a common stage in the genesis of circulatory disorders followed by various pathologies.

**Hydratation and dehydratation of the vascular wall**

In normal tissues, extracellular (matrix, interstitium) and juxtacellular (glycocalyx) gel components are maintained under relatively dehydrated conditions [27]. This facilitates proper circulation and vascular permeability. Hydration volume of gel matrix is regulated by equilibrium of various forces, including elasticity of polymer components, their chemical affinity, fixed charge, and osmotic interactions, being dependent on glycosaminoglycans, i.e., mechanical and structural factors responsible for their content and spatial distribution in intact tissues.

Macromolecular interactions which control and determine atherogenesis are aimed against dehydration forces that prevail in vivo in the norm. Indeed, shift of the dehydration/hydration equilibrium towards hydration occurs upon glycocalyx destruction caused by various pathological factors (Figure 3) [28,29]. This, in turn, affects hydration volume, modifying glycosaminoglycan structure and distribution [27]. Glycocalyx integrity plays an important role in tissue protection against edema. Under pathological conditions (inflammation, thrombosis, infection, etc.) hydration manifests itself as edema of arterial tissues, which hampers the flow of nutrients and drugs [28]. Diffusional thickening and fibril disorganization in the intima occurring in atherosclerosis is associated with changes in local water homeostasis [27].

These findings show that dehydration and hydration of endothelial glycocalyx are important parameter of normal and deceased vascular wall. This is supported by the fact that the body of an adult with good cardiopulmonary health consists of 60% water [30]. Two thirds of this volume is localized intracellularly, one third, extracellularly. Interstitium contains 80% of this volume; other 20% is part of blood plasma volume.

**Water-sulfate exchange**

Sulfation of glycosaminoglycans is a specific factor affecting water balance in the glycocalyx. Alterations in this parameter are associated with the volume of solvent available/required for hydration [27,31]. Hydration of chondroitin sulfate increases with progression of atherosclerotic lesions, which decreases its anticoagulant activity (antithrombin activation) [26] and increases glycocalyx hydration. Insertion of $^{35}$SO$_4$ in glycosaminoglycans of chicken glycocalyx was slowly (12 h) suppressed by the tetrasaccharide hyaluronan and its derivatives of higher molecular mass [32]. Total evidence indicates that in blood vessels with atherosclerotic plaques sulfate insertion is linked to accumulation of sulfated glycosaminoglycans in the atheroma for their redistribution. This can be regarded as an indirect preparation for an increase in vascular area with low sulfation suitable for a potential lesion [33]. Sulfated glycosaminoglycans maintain zones of structured water which are necessary for normal circulation (condition of the endothelium and motion of red blood cells along capillaries). Sulfur deficiency in the body can contribute to a decrease in cholesterol sulfate level and glycosaminoglycan sulfation [33]. Loss of sulfates provokes changes in structural water that lead to accumulation of cholesterol in atheroma, since its transport in water-based media depends on sulfation, i.e., cholesterol transport disorders are related to low bioavailability of sulfate in the circulation. It has been hypothesized that low cholesterol sulfate content together with decreased sulfation of glycosaminoglycans in the body is a key lesion propulsive to atherosclerosis progression [33].

**Water-sodium exchange**

Negative charge on the vascular wall (created predominantly by...
Glycocalyx and reactive oxygen species

The multiform interaction between systemic factors such as endothelial glycocalyx and oxidative stress in blood circulation emphasizes and grounds the signification and perspective for consecutive development of novel prevention and therapy approaches for sustention and cure of cardiovascular human health.

Glycocalyx is involved in oxygen metabolism in the circulatory system. At moderate concentrations reactive oxygen species (ROS) participate in cell signal transduction, while at higher concentrations they produce damaging effects [38]. Endothelial dysfunction as a key initial stage in the development of atherosclerosis can be associated with high ROS production or oxidative stress which is a major pathogenic factor of this process. A common mechanism of damaging effect renders oxidative stress a dominating factor in the development of various cardiovascular disorders [39,40]. Excessive ROS generation leads to destruction of endothelial glycocalyx [41]. To prevent and reduce this damage the glycocalyx accumulates antioxidants e.g., extracellular superoxide dismutase (exSOD, Figure 4). This enzyme has high affinity for heparansulfates of endothelial glycocalyx due to a specific positively charged binding domain for heparin-like compounds and high expression (up to 70% total vascular superoxide dismutase / SOD/ content) [11]. It was reported that glycocalyx has a role in NO and ROS production [42]. Ex vivo incubation of femoral porcine arteries with hyaluronidase to remove hyaluronan decreased both nitrite content and vasodilation. Degradation of heparansulfateproteoglycan and syalic acid by heparinase III and neuraminidase, respectively, reduced NO bioavailability by increasing superoxide production. This finding indicates that hyaluronan is involved in shear stress-induced NO production and heparansulfate together with syalic acid participates in ROS production in response to shear stress. Since a vast...
Figure 4. A hypothetic scheme showing the major glycocalyx components on the endothelial surface. Location of extracellular superoxide dismutase (SOD) which displays affinity for endothelial heparan sulfate structures is indicated. Heparan sulfate fulfills protective function by accumulating antioxidants, including extracellular SOD which converts cytotoxic superoxide radical into hydrogen peroxide.
majority of cardiovascular disorders is associated with oxidative stress, its damaging effects should be blocked or reduced. Endogenous and exogenous antioxidants can be employed for this purpose.

**Formation of antioxidant therapy**

Clinical studies of antioxidants test the hypotheses that low contents of glutathione peroxidase and superoxide dismutase increase the risk of major adverse cardiovascular events [43], modified expression and/or activity of antioxidant enzymes is associated with oxidative stress which is an important factor in the pathogenesis of age-related (macular degeneration) and degenerative diseases [44], as well as evaluate the effectiveness of antioxidant therapy and identify unfavorable effects of oxidative stress on specific clinical parameters of cardiovascular therapy [39,40].

Formation of antioxidant therapy implies investigation and development of new antioxidant drugs [45,46]. Protective effects of ascorbic acid, trolox, melatonin, polyphenols [47], human recombinant Mn-SOD [48] in hepatic disorders, the effects of chemically modified SOD in lipid oxidation and antioxidant status in diabetic rats [49], changes in SOD and catalase (CAT) activities due to cadmium-induced incorrect folding [50] have been examined. Antioxidant enzymes remain an important object of biomedical research.

Superoxide dismutase and catalase (up to 30% total content) were precipitated on a protective antioxidant nanocarrier for endothelial targeting and bound to antibodies against endothelial adhesion molecules to provide the targeting [51]. In a mouse model of endotoxin-induced pulmonary damage CAT derivative protected endothelium, decreased lung edema and leukocyte infiltration. SOD derivative reduced cytokine-induced proinflammatory activation of the endothelium and endotoxin-induced pneumonia. A modular approach has been suggested for targeting therapeutic enzymes at the vascular wall [51]. Among three concentration intervals of reactive forms of oxygen and nitrogen species (physiological, enhanced and toxic), there is the favourable effect on toxic interval with such approaches as center-specific, nanocarrier-based therapy [52], 3D supramolecular ensembles with antioxidants or enzymes [53] and nanotherapeutics with an antioxidant nucleus for blocking atherogenic oxidative stress ensambles with antioxidants or enzymes [53] and nanotherapeutics as center-specific, nanocarrier-based therapy [52], 3D supramolecular ensembles with antioxidants or enzymes [53] and nanotherapeutics as center-specific, nanocarrier-based therapy [52].

**Bienzayme conjugate of antioxidant biocatalysts**

Covalent binding of superoxide dismutase and catalase via chondroitin sulfate in the SOD-CHS-CAT conjugate provides a simultaneous action of these enzymes in the damaged area. The antithrombotic effect in vivo produced by the conjugate is higher than those of various combinations and mixtures of its components (native or modified) [46,56], is achieved at low doses (25-50 U SOD and 55-110 U CAT per rat), which is one or two orders of magnitude lower than those for native or modified enzymes [57]. Chondroitin sulfate content on the vascular surface over atherosclerotic lesions increases at the initial stages of atherogenesis [58]. Intimal thickening is associated with the presence of versican, a proteoglycan with chondroitin sulfate and dermatan sulfate chains [59]. Versican is accumulated in the neointima of human blood vessels prone to atherosclerosis [60]. Specific features of chondroitin sulfate [58-60] render it into a productive linking agent for antioxidant enzymes [45] and facilitate targeting of the resulting conjugates at the glycosalyx over vascular lesions. This may account for the effectiveness of SOD-CHS-CAT conjugate at small doses [45,46]. It should be noted that in an animal model of endotoxic shock SOD-CHS-CAT significantly increases viability after preventive and therapeutic administration [56]. These results are interesting in comparison with modeled therapeutic effects of modular approaches [51] and for a better understanding of the mechanism underlying the conjugate activity involving NO-dependent and NO-independent pathways for its therapeutic effect. The effectiveness of SOD-CHS-CAT conjugate urges further development of genetic and bioengineering technologies for production of three-functional enzymes with activities of SOD and CAT [61] and peroxidase and SOD [62] capable of entering the cell.

Damaging effect of oxidative stress on the vascular wall is determined by a complex of multivariate factors. An array of variables should be taken into account to evaluate and control this effect, which actualizes the use of computational methods to optimize the solution of the specific biomedical problems.

**Computational studies of systemic factors and their regulators**

Calculated interactions (computational models) have been used for several decades to evaluate and predict function of biological systems, e.g., fibrinolysis, coagulation, etc. This approach, however, implies a complex processing and presentation of the data obtained with a wide experimental verification gap. Nevertheless, the significance of computational methods increases as new systemic factors are considered for achieving the goals set up by translational medicine. Current methods are oriented toward experimental verification and cover general objects of analyzed systems. Variations in the microcirculation were studied using modeled glycosalyx-endothelium-erythrocyte interactions [63]. These variations are determined by vascular wall modifications associated with endothelial cell shape, glycosalyx-related effects and specific parameters of the blood. Blood is modeled as a two-component (plasma and corpuscular elements) incompressible fluid. Endothelial glycosalyx is modelled as a medium of variable and adaptive porosity manifesting itself in the magnitude and temporal variations of blood flow rate and its shear stress. Combined and simultaneous effect of endothelial wall undulation, glycosalyx compression and repulsion, and specific nature of the blood on the flow properties has been demonstrated [63].

Structural reconstructions of components and external agents that can influence biological system have been performed to analyze the state of the vascular wall. Investigation of the mechanism responsible for specific interactions between glycans-binding protein with cognate glycan from cell glycane array (glycome) was initiated with the use of automated 3D structure generation technique (computational carbohydrate grafting) [64]. Integration of extensive data from glycans screening and protein crystallography facilitates construction of putative co-complex structures that can be objectively assessed and iteratively altered until a high level of agreement with experiment is achieved. This approach allows differentiation of active glycans determinants from potential ones. When applied to a collection of 10 protein-glycan complexes, for which crystallographic and array data have been reported, grafting provided structural rationalization for the binding specificity of >90% of 1223 arrayed glycans [64]. Automated molecular docking and interaction mapping techniques were employed to characterize glycosaminoglycan-protein interactions [65]. The method was used for identification of glycosaminoglycans...
GAG capable of binding to acidic fibroblast growth factor. The results obtained demonstrate the value of mapping-based techniques in identifying specific GAG epitopes recognized by proteins and for GAG-based drug design. Combinatorial virtual library screening technology was used to predict high-specificity GAG sequences, bearing in mind their phenomenal structural diversity [66]. The results highlight critical interactions in heparin/heparan sulfate oligosaccharides that regulate specificity, the minimal specificity element being a disaccharide of heparin.

The antioxidant enzymes catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase act as defense agents against oxidative stress. Three-dimensional structures of these enzymes were modeled and docked with nonylphenol and bisphenol A which induce oxidative stress [67]. The study revealed that the enzymes have binding sites for nonylphenol and bisphenol. Maximum inhibition by nonylphenol was demonstrated for catalase and by bisphenol A for superoxide dismutase. These findings specify the conditions for the use of SOD and CAT. Microcirculation disturbances can be controlled by modifying the state of the glyocalyx with enzymes, for example, hyaluronidase [68]. Effects of hyaluronidase in native and chondroitin sulfate modified forms demonstrated the regulation potential for microcirculation recovery after ischemia/reperfusion tracked using laser Doppler flowmetry. This action is actual for cardiovascular therapy of acute myocardial infarction, thromboses, no-reflow [69]. However, scarce information about the structure of these biocatalysts hampers the research into regulation of the glyocalyx state. To solve this issue a 3D-model of bovine testicular hyaluronidase (BTH) [70] based on established tertiary structure of human hyaluronidase [71] was constructed using a molecular homological modeling method in silico (Figure 5). Superposition of BTH with human and bee venom hyaluronidases has revealed differences between these enzymes [70].

Human hyaluronidase has a Ser-353 – Trp-435 C-terminal domain homologous to epidermal growth factor [71] which is present in our model of BTH (Figure 5) and bee venom hyaluronidases. Epidermal growth factor domain has three disulphide bonds, is essentially stable, regulates binding with other proteins and modulates the activity of a full-size enzyme [70,71]. A 3-D structural model of chondroitin-modified BTH was constructed by modeling covalent binding between lysine residues of the enzyme and benzoquinone-activated chondroitin sulfate links (Figure 6). The significance of deep modifications of BTH for production of its active and stable derivatives was demonstrated by varying the degree of the enzyme modification and the size of covalently bound chondroitin sulfate chains and was confirmed experimentally [70]. Effective size of chondroitin sulfate coating is achieved with 180-kDa enzyme derivatives obtained with the use of 30-50-kDa and/or 120-140-kDa chondroitin sulfate. Lysine residues on the surface of BTH have different bioavailability: there are external free and semifree residues (their modification affects catalytic domain) and cryptic residues [70]. It should be noted that epiphycan effectively binds to lysine residues of type I, III, VII, VIII and X collagen via clustered chondroitin sulfate, while the affinity of binding to type II, IV, V, VI and IX collagen displays low affinity [72]. Chemical modification of collagen lysine residues decreases binding affinity for clustered chondroitin sulfate and determines binding affinity for epiphycan. Examination of a 3-D modeled initial stage of docking of BTH with chondroitin sulfate trimer or heparin tetramer revealed several surface binding sites for GAG on the enzyme molecule. Both reversible and irreversible conformational changes depending on location of other negatively charged GAG ligands on the protein globule were demonstrated. In irreversible conformational changes, Glu-149 (E 149) and Asp-147 (D 147) residues that play a key role in enzyme activity move from the active center to the periphery, which causes inactivation of the enzyme (Figure 7). These findings suggest...
Figure 6. Illustrated visualization of 3-D model of bovine testicular hyaluronidase covalently modified by two chondroitin sulfate chains (designated as CHS and highlighted gray, red and brown). The chains are necessary for deep modification of the enzyme with binding at its 19 lysine residues (positions 77, 106, 122, 129, 176, 187, 190, 198, 206, 244, 255, 271, 292, 392, 416, 430, 446, 447). Enzyme structure shown as a ribbon sort and as segment forms is masked by covalently bound chondroitin sulfate chains (30 kDa).

Figure 7. An irreversible conformational change in bovine testicular hyaluronidase with Glu-149/E 149 and Asp-147/D 147 moved to the periphery of enzyme structure and considerable alterations in protein loop/active center chain (highlighted violet). Location of enzyme structures on the 40th psec (beige) and on the 160th psec (blue) of molecular dynamics induced by glycosaminoglycan ligands.
some threshold interactions and set the goal of their identification to obtain control over hyaluronidase function on the vascular wall.

Presented above data have been showed the alterations of our knowledge in respect to functioning of multicomponent biological systems with application of computational methods and with consequent ascertainment of approaches for regulation of their state.

Conclusion and future perspectives

Extensive systematic studies of the glycocalyx have revealed specific factors limiting its function. Better understanding of the mechanisms responsible for regulation of glycocalyx hydration opens new perspectives in the study of initial vascular wall damage related to water-electrolyte balance disorders. Further progress in this study is determined by clinical data. Control over the interaction of factors in extensive systems such as vascular glycocalyx and oxidative stress offers productive approaches in antioxidant therapy and highlights the role of antioxidant enzymes in correction of metabolic disorders. Computational methods for multicomponent systems proved to be useful for therapeutic regulation of the vascular system function and provide a better understanding of the principles of endothelial glycocalyx function with the development of approaches to their effective use. Experimental findings largely determine progress in this direction in largely: Fundamental insight into molecular and cellular mechanisms operating in the vascular system could contribute to translational cardiology. Clinical and biochemical research into vascular wall hydration and water electrolyte balance provides new insights in the mechanisms underlying the initiation of vascular lesions, their prevention and therapy. Consecutive study of the glycocalyx function, computational research of its relationship with oxidative stress, and investigation of the glycocalyx state regulation is aimed at the development of novel approaches to the vascular wall protection. Successful development of translational medicine depends on close cooperation between scientists and clinicians as well as on constantly increasing data flow from life science laboratories to clinics and vice versa. This crosswind move is a determinant factor of efficacy for translational cardiology.

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