Transport and biomechanical studies in the isolated blood vessel segment

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Abstract

Experiments on the isolated blood vessels are very important for investigation of drug effects, new drug development, blood vessels regulatory mechanisms and other functional and biomechanical investigations. In this paper we designed an experimental study and mathematical model for dynamics response of blood vessel under different load conditions. The results from these experiments demonstrated influence of shear stress dynamics and other biomechanical properties of the blood vessel. Also the effects of drugs are different even they produce the same relaxation or constriction at alternate steady states. Experimental data for different shear stress-strain curves were fitted using exponential mathematical function. This research is very important for biomechanical analysis of blood vessels during dynamics loads.

**Key words:** Transport study, biomechanical analyses, isolated blood vessel segment

1. Biomechanical studies

Ex vivo blood vessels experiments in numerous studies [Ligush et al. 1992], [Madden et al 1999], [Marra et al. 2006], [Jerius et al. 2000], [Plane et al. 1998], [Gonzales et al. 2001], [Sofola et al. 2004], [Leone et al. 2004] have been commonly performed in strips or rings of blood vessels as well as in the isolated perfused blood vessels segments.

All mentioned experimental methods were used for various functional investigation (drug effects on smooth muscle, regulatory mechanisms in control of vascular tonicity, etc.) and biomechanical investigations (uniaxial or biaxial stretch, to obtain stress-strain relations and other biomechanical parameters).

Each experimental design has been created to provide some specific information such as: relaxation or constriction of blood vessels smooth muscle under different conditions, or passive and/or active biomechanical properties of blood vessels.

In various studies, only the time end points of the dynamic response have been considered, or the so called the "alternate steady states" of the processes (Fig. 1). By considering the end points only, we do not have an insight into the processes variables between these alternate steady states. The behavior of blood vessels between the alternate steady states is referred as the transient or dynamic response.
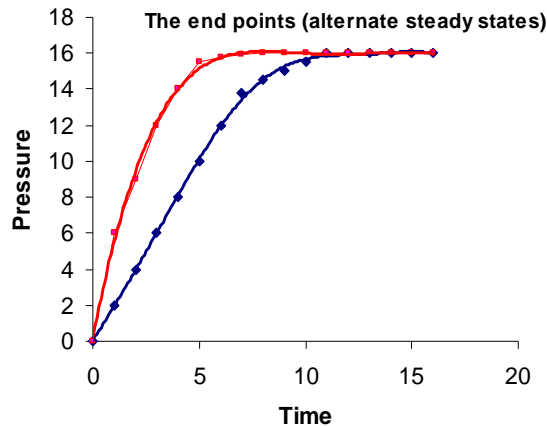
Fig. 1. The "end points" (alternate steady states) of blood vessel response to some drugs could be the same, but dynamic responses could be different. This will lead to different dynamic of shear stress and other biomechanical properties of the blood vessel, and the effects of these drugs will be different even they produce the same relaxation or constriction at alternate steady states.

In our studies [Rosic et al. 2006], [Rosic et al. 2008], [Pantovic et al. 2009], [Kostic et al. 1995] we use a new approach to *ex vivo* (isolated perfused blood vessel segments) experiments in which not only the end points of vessels response within the time interval of dynamic loading is considered, but also dynamics of this response. Further, the functional and biomechanical investigations could be performed at the same time by means of single experimental design.

This experimental method is created to measure the changes in pressure and vessel diameter when an abrupt change of pressure occurs at the vessel outlet, by applying various hydrostatic levels or resistance as step functions.

We evaluate the time history of the pressure and the vessel diameter changes in between alternate steady states, represented by the pressure-time and diameter-time curves.

Intraluminal pressure at the constant perfusion flow and changes in blood vessels diameter, under different hydrostatic pressure conditions in the isolated blood wessel, were measured by means of the System for Biomechanical and Functional Tissue Investigations (Fig. 2)

The System for Biomechanical and Functional Tissue Investigations allows:

- Continual recording of pressure and/or force developed during various design of experiments (electro stimulation, drug effects, stretch, etc.) by mechanoelectrical transducer and/or pressure transducer (by applying various hydrostatic levels at the vessel outlet);

- Continual recording of changes in blood vessel outer diameter during experiment;

- Continual addition of drugs into the perfusion medium by micro infusion pump, for drug effects investigations;

- Linear or nonlinear fitting of experimental data (pressure and blood vessel diameter) with various mathematical formulas;

- Statistical analysis of experimental data.
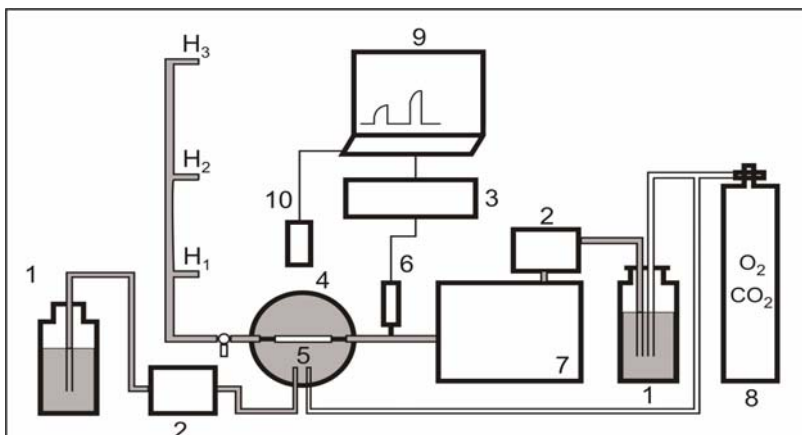
Fig. 2. Schematic diagram of the System for Biomechanical and Functional Tissue Investigations (1. perfusion fluid; 2. peristaltic pump; 3. AD converter and amplifier; 4. organ bath; 5. blood vessel; 6. transducer; 7. water bath; 8. oxygen bottle; 9. PC; 10. Camera; 11. H0, H1, H2, H3 hydrostatic levels.)

Experimental data as the pressure/time curves were fitted using an exponential mathematical function:

$$y = b_1\left(1 - e^{-b_2 x}\right)$$

(1)

where y is the pressure (in mmHg) and x is the time (s). Also, $b1$ and $b2$ are the coefficients of the curve: b_1 has units of pressure, and b_2 has units of time^{-1}.

The dominant time constant follows from the function (1): $T = 1/b_2$. We consider that the alternate steady state is reached for $t=5T$ (t=time).

To define dynamic properties of the blood vessels, we introduce a coefficient of the comparative pressure dynamics (Pd)

The coefficient of comparative pressure dynamics (Pd) is defined as the integral of the difference between two fitted curves, normalized with respect to the applied hydrostatic pressure (H):

$$P_d = \frac{1}{H}\int_0^\infty \left\{\left[b_1\left(1 - e^{-b_{2b}x}\right)\right] - \left[b_1\left(1 - e^{-b_{2a}x}\right)\right]\right\}dx$$

(2)

where b_{2a} is the coefficient of the first curve (control curve) and b_{2b} is the coefficient of the second curve (test curve –in the presence of some investigated drug or stimuli). Here b_1 is considered the same for booth function under the integral. The solution of the equation (2) is:

$$P_d = \frac{b_1}{H}\frac{b_{2b} - b_{2a}}{b_{2a} \times b_{2b}}$$

(3)

The calculated P_d is the area between the test and control curves and it is normalized to become a dimensionless quantity. Positive value of P_d indicates the shift of the test curve to the

left and faster development of maximal pressure (alternate steady state). In contrary, negative value of the P_d indicate the shift of the test curve to the right and slower development of maximal pressure.

We also introduce a coefficient of total pressure dynamics (TP_d) as the area under the test and/or control curves which can be calculated from the equation:

$$TP_d = b_1 \left(x + \frac{e^{-b_2 x}}{b_2} \right) - \frac{b_1}{b_2}$$

(4)

This coefficient is useful for comparing the results obtained from separate experimental group (treated and untreated animals; males and females etc.)

Experimental data, presented as the diameter/time curves, were fitted using exponential mathematical function (1), as described in the previous section, but in this case y is the blood vessel diameter (mm) and x is the time (s). Here, b_1 and b_2 are the coefficients of the curve: b_1 has units of diameter and b_2 has units of time^{-1}.

The constant b_1 now represents the maximum diameter, when the experimental curve approximately reaches the value of the alternate steady state.

As in the previous protocol we consider that alternate steady state is established for $t=5T$.

Analogously to the expression (2), we define distensibility properties of the blood vessels by the coefficient of comparative distensibility D_k:

$$D_k = \frac{1}{D_0} \int_0^\infty \left\{ \left[b_1 \left(1 - e^{-b_{2b} x} \right) \right] - \left[b_1 \left(1 - e^{-b_{2a} x} \right) \right] \right\} dx$$

(5)

where D_0 is the original vessel diameter. The meaning of the coefficients b_1, b_{2a} and b_{2b} are as for the relationship 7. Further, we have:

$$D_k = \frac{b_1}{D} \frac{b_{2b} - b_{2a}}{b_{2a} \times b_{2b}}$$

(6)

Positive values of D_k indicate faster development of maximal diameter (alternate steady state), while negative values indicate slower development of maximal diameter.

Continual recording of pressure and diameter during experiments allows us to evaluate the stress and strain according to the following relations:

$$\sigma_t = P_t \frac{R_t}{\delta_t}$$

(7)

where, σ - stress [kPa]; P - pressure; R- blood vessel internal radius; δ - blood vessel wall thickness; and

$$\varepsilon_t = \frac{R_{mt} - R_{m0}}{R_{m0}}$$

(8)

where, ε - circular strain; R_m - blood vessel mean radius; R_{m0} - blood vessel mean radius at the time zero (original mean radius). Here, the right lower index t indicates the values at time t.

The calculated values are than presented as the stress-strain curves and fitted using a nonlinear (polynomial) mathematical function.

Assuming that the blood vessel wall is incompressible, we can determine the wall thickness for the current mean radius

$$\delta_t = R_{mt} - \sqrt{R_{mt}^2 - \left(\frac{V_0}{\pi L}\right)}$$

(9)

where, L - length of the isolated blood vessel; and V_0 is the original volume of the blood vessel,

$$V_0 = \pi (R_0 - \delta_0) \delta_0 L_0$$

(10)

where, the sub index „0" indicates the original values.

The wall shear stress (kPa) can also be calculated from the following equation:

$$\tau_t = \frac{4\mu Q}{\pi R_t^3}$$

(11)

where τ - shear stress (kPa); μ - viscosity (poise); Q - flow (ml/s);

The calculated values are then presented as the shear stress–strain curves and fitted using nonlinear (polynomial) mathematical function.

2. Rapid dual –isotope dilution method

The transport of a radiolabeled test molecule can be investigated by means of rapid dual-isotope dilution technique using the same experimental setting described above (Fig.2).

This technique measures the relative extraction of the test molecule in relation to mannitol, (as an extracellular reference tracer), or radiolabeled albumin (as an intravascular reference tracer), as it transits the blood vessel segment.

The rapid dual-isotope dilution technique allows calculation of following transport parameters [Kostic et al 1995], [Rosic et al. 1998], [Rosic et al. 2001]: maximal cellular uptake (Umax), net cellular uptake (Unet), backflux (BF), fractional and maximal extraction (Ef and Emax) from intravascular compartment, as well as transport capacity (Vmax) and Michaelis-Menten (affinity) constant (Km) for carrier mediated transports.

After the blood vessel have been excised and perfused with a peristaltic pump using the constant-flow   technique, a bolus containing ^{3}H test molecule and D-^{14}C manitol as an extracellular reference tracer or ^{14}C albumin as an intravascular reference tracer have been injected into the perfusion system.

The effluent samples have been sequentially collected and than prepared for counting in a Rackbeta scintillation counter.The activity of each isotope recovered in the effluent sample, in desintegrations per minute (d.p.m.), has been than ploted against collection time or sample number in order to obtain concentration-time curves, i.e. dilution profiles for both test and reference traces.

The difference in radioactivity between the value of reference molecules and test molecule suggests an uptake (extraction) of test molecule.

The cellular uptake of test molecule have been estimated directly from the dilution profiles using eq. (12), where ^{3}H test and D-[^{14}C]mannitol represent activities (in d.p.m.) recovered in successive effluent samples.

$$\text{Uptake (\%)} = (1 - [^3H] \text{ test} / D-[^{14}C] \text{ mannitol}) \, 100 \qquad (12)$$

If we use albumin instead of mannitol as a reference tracer, than calculated value from eq. (12) will represent extraction of test molecule from intravascular to extravascular space.

The maximal uptake (*U*max*)* for test molecule is the mean of the uptake values taken over the time period when the uptake has reached a plateau.

The *Unet* is the mean uptake over the whole period, i.e. the recovered activity in all samples. The net cellular uptake (*Unet)* can be calculated as:

$$\text{Unet} = (1 - \text{Total recovered } ^{14}C / \text{Total recovered } ^3H) \, 100 \qquad (13)$$

The backflux (BF) of test molecule (from intracellular to extracellular space or from interstitial to intravascular space) can be calculated as:

$$\text{BF} = (\text{Umax} - \text{Unet}) / \text{Umax} \qquad (14)$$

The unidirectional transport or flux of test molecule has been estimated (in mmol min-1 g-1) from the maximal fractional tracer uptake (Umax), the perfusion rate (F, ml min-1) and concentration of unlabelled test molecule in perfusate (Ca,μM):

$$V = -Fln \, (1 - Umax)Ca \qquad (15)$$

Linear increase of V indicates nonsaturable transport (simple diffusion) while exponential increase indicates carrier mediated transport.

From the rate of test molecule uptake at different concentrations of unlabelled test molecule, the maximal rate of transport (Vmax) and Michaelis-Menten constant (Km) can be calculated using Eadie-Hofstee analysis if the transport is carrier mediated.

3. Concluding remarks

We believe that described experimental design and applied mathematical procedures, could be a powerful tool in further investigations of: drug effects, new drug development, blood vessels regulatory mechanisms and other functional and biomechanical investigations, including computational modeling dynamic behavior of blood vessels and molecular transport in the blood vessel wall.

Извод

Биомеханичка анлиза и транспортна студија на изолованом крвном суду

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Резиме

Експерименти на изолованим крвним судовима су веома значајни за испитивање ефекта дејства и развоја нових лекова, регулаторног механизма и осталих функционалних и биомеханичких истраживања. У овом раду је дизајнирано експериментално истраживање и математички модел за динамички одзив крвних судова под различитим оптерећењем. Резултати ових експеримената показују утицај динамике, смичућег напона и биомеханичких карактеристика крвних судова. Такође је показано да су ефекти коришћења лекова различити иако производе исту релаксацију и сужење при промени стационарног стања. Експериментални подаци за различите дијаграме смичућег напона и деформације су фитоване помоћу математичких функција. Ово истраживање је веома значајно за биомеханичку анализу крвних судова за време динамичког оптерећења.

Кључне речи: Транспортна студија, биомеханичка студија, изоловани крвни суд

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