Dynamic response of blood vessel in acute renal failure

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Abstract. In this study we postulated that during acute renal failure induced by gentamicin the transient or dynamic response of blood vessels could be affected, and that antioxidants can prevent the changes in dynamic responses of blood vessels. The new approach to ex vivo blood vessel experiments in which not only the end points of vessels response within the time interval is considered, but also dynamics of this response, was used in this paper. Our results confirm the alteration in dynamic response of blood vessels during the change of pressure in gentamicin-treated animals. The beneficial effects of vitamin C administration to gentamicin-treated animals are also confirmed through: lower level of blood urea and creatinine and higher level of potassium. The pressure dynamic responses of isolated blood vessels show a faster pressure change in gentamicin-treated animals (8.07 ± 1.7 s vs. 5.64 ± 0.18 s). Vitamin C administration induced slowdown of pressure change back to the control values. The pressure dynamic properties, quantitatively defined by comparative pressure dynamic and total pressure dynamic, confirm the alteration in dynamic response of blood vessels during the change of pressure in gentamicin-treated animals and beneficial effects of vitamin C administration.

Key words: Blood vessel dynamic response — Gentamicin-nephrotoxicity — Vitamin C

Introduction

The aminoglykoside antibiotic, gentamicin, is well known to cause renal failure. Gentamicin-induced nephrotoxicity is a complex phenomenon leading to morphological and structural alterations of glomeruli and glomerular basement membrane as well as alterations of proximal tubules (Bennet 1986; Can et al. 2000; Stojiljkovic et al. 2008). The molecular mechanisms underlying gentamicin-induced renal failure have not been fully elucidated although superoxide production (Kays et al. 1991; Ben Ismail et al. 1994; Ata Secilmis et al. 2005), alteration in lysosomal enzymes (Olbricht et al. 1991) and inhibition of microsomal protein synthesis (Bennet et al. 1988) have been proposed to contribute to its deleterious effect.

The well known pattern of gentamicin-induced nephrotoxicity is characterized by reduction in glomerular filtration rate and consequent reduction in creatine clearance with increased serum creatinine. The plasma urea nitrogen is also increased as well as fractional excretion of Na+ and Li+. After 5 days of gentamicin administration to rats (100 mg·kg–1), unchanged blood pressure has been reported (Rivas-Cabero et al. 1995; Can et al. 2000), but after 9 days of gentamicin administration to rats, significant increase in systolic blood pressure has been reported (Can et al. 2000). Recent investigations (Kingma et al. 2006) suggest that coronary vascular tone, reserve and vessel reactivity are markedly diminished in dogs with acute renal failure (ARF), suggesting impaired vascular function. It is also known that advanced oxidation protein products (AOPP) act as mediator of oxidative stress and monocyte respiratory burst, which points to monocytes as both target and actor in the immune dysregulation associated with uremia (Witko-Sarsat et al. 1998). Furthermore, it has been demonstrated that AOPP can induce the impairment of endothelium-dependent relaxation in isolated rat aorta rings, which may be partly due to decrease in NO production and/or release and increase in oxygen free radicals (Shuangxiu et al. 2005). Additionally, exposure to urea, which is elevated in renal failure, leads to the carbamilation
of proteins (Kraus and Kraus 2001). Recent investigations (Ok et al. 2005) demonstrated that carbamylated low-density lipoprotein (cLDL) induced dose-dependent vascular cell injuries which included the proliferation of vascular smooth muscle and endothelial cell death.

Impaired vascular function in ARF and possible molecular mechanisms underlying this impairment have been investigated by means of various experimental methods. Only the end points within the time interval of the blood vessels response, or so-called “alternate steady states” of the processes, were usually considered in these studies. By considering the end points only, we do not have an insight into the process variables between these alternate steady states. The behavior of a blood vessel between the alternate steady states is referred as the transient or dynamic response. In our previous work (Rosic et al. 2008) we developed the experimental model and adequate mathematical procedures, which can be used to describe dynamic response of isolated blood vessels to different stimuli.

In this study we postulated that the transient or dynamic response of blood vessels could be affected during ARF. We also postulated that antioxidants can prevent the changes in dynamic responses of blood vessels in ARF, because all mentioned molecular mechanisms underlying impaired vascular function in ARF (such as AOPP, cLDL, NO, …) are based on increase in oxygen free radicals production. In as much, the recent evidence suggests protective effects of vitamin C on gentamicin-induced nephrotoxicity (Kadkhodaee et al. 2005, 2007).

Materials and Methods

The total number of 35 animals was divided into 3 groups, one of which was used as a sham control. The control group of animals (C-group) received 1 ml/day saline intraperitoneally. The first experimental group (GM-group) of animals received gentamicin (Galenika AD, Belgrade, Serbia) intraperitoneally in a daily dose of 100 mg·kg⁻¹. The second experimental group (GMC-group) of animals received gentamicin as the GM-group, and vitamin C in a daily dose of 50 mg. The experimental and control groups were treated over the period of 8 consecutive days. Following the last application, that is 9 days after the beginning of the experiment, all animals were sacrificed. Immediately, 2 ml blood was taken from the aorta for biochemical analysis. Plasma creatinine, blood urea, sodium and potassium concentrations were measured using an automatic biochemical analyzer (A25 biosystem, Barcelona, Spain).

Intraluminal pressure at the constant perfusion flow under different hydrostatic pressure conditions in the isolated perfused blood vessels segments were measured by means of the System for Biomechanical and Functional Tissue Investigations, described in details in our previous work (Rosic et al. 2008).

The pressure transducer is placed at the inlet of the blood vessel. The outlet of the vessel is connected to the two-way tap attached to two tubes filled with perfusion solution, allowing a change of hydrostatic pressure, from \(H_0\) (0 mmHg) to \(H_1\) (60 mmHg), as a step function. When an abrupt change of hydrostatic pressure from \(H_0\) to \(H_1\) occurs, the pressure wave propagates backward and is detected by the pressure transducer. The pressure change detected by transducer depends on elastic properties of the segment between the pressure transducer and two-way tap. Because the only elastic part of this segment is blood vessel (the rest of the segment are rigid tubes) we can assume that the dynamics of pressure change depends only on elastic properties of the blood vessel for an applied hydrostatic level.

Experimental procedure

All experiments were performed according to EU (86/609/EEC) and local ethical guidelines. Wistar rats of both sexes (200–250 g body weight) were killed by cervical dislocation. The segment of the abdominal aorta was rapidly isolated and transferred to the water bath. Glass cannulas with equally matched tip diameters are mounted at proximal (inflow) and distal (outflow) ends of the blood vessel. The proximal end of the artery is tied at the place on the proximal cannula with a silk thread, and the lumen is perfused with Krebs-Ringer physiological solution (KRS), using a peristaltic pump at 9 ml·min⁻¹. The perfusate was continuously bubbled with a 95% \(O_2\) and 5% \(CO_2\), with the pH adjusted to 7.4 at 37°C. The distal end of the artery is then tied onto the distal cannula. The distal cannula was connected to the two-way tap attached to two tubes filled with perfusion solution, allowing a change of hydrostatic pressure from \(H_0\) (0 mmHg) to \(H_1\) (about 60 mmHg). The exterior of the vessel was also perfused with KRS from a reservoir using a peristaltic pump at 3 ml·min⁻¹, on 37°C and aerated with the same gas mixture as the lumen of artery.

The artery was stretched to its approximate in vivo length using a micrometer (2–2.5 cm). The total time duration of our experiments was within the experimental time in the most frequently used procedures on the isolated blood vessels.

Experimental protocol

As described above, the blood vessels segment was dissected from the rat abdominal aorta and placed into the water bath. Following the equilibration period (20–30 min), the segment was treated by increasing hydrostatic pressure from \(H_0\) to \(H_1\). This protocol was repeated 3–5 times for each segment \((n = 5)\) and a resting period of several minutes (usually 15
min) was allowed between two activities. The development of pressure was recorded on a computer, continuously using the system described above.

After this, we added acetylcholine (final molar concentrations in the perfusion fluid were 10 μmol l⁻¹) continuously into the perfusion system with micro infusion pump at 100 μl min⁻¹. The same protocol (in the presence of acetylcholine) from H₀ to H₁ hydrostatic pressure at constant perfusion flow was repeated 3–5 (n = 5).

Experimentally recorded dependence of pressure on time was fitted using an exponential mathematical function:

\[ y = b₁ \left( 1 - e^{-b₂x} \right) \] (1)

where \( y \) is the pressure (in mmHg) and \( x \) is the time (in seconds). Also, \( b₁ \) and \( b₂ \) are the coefficients of this relationship: \( b₁ \) has units of pressure, and \( b₂ \) has units of \( T^{-1} \).

This function is shown in Fig. 1A as the pressure vs. time curve. The constant \( b₁ \) represents the maximum developed pressure, i.e. the pressure corresponding to the alternate steady state.

We introduce a dominant time constant (\( T \)) as the time value corresponding to the cross section point between the asymptote of the exponential curve and the tangent of the exponential curve at the zero point. This constant follows from the Eq. (1):

\[ T = 1/b₂ \] (2)

We consider that the alternate steady state is reached for time \( t = 5T \), because in this case, \( e^{-5T} \approx 0 \), and \( y = b₁ \).

Then, to define dynamic properties of the blood vessels, we introduced a parameter of the comparative pressure dynamics (\( P_d \)). This parameter is defined as the integral of the difference between two fitted curves, normalized with respect to the applied hydrostatic pressure (\( H \)):

\[ P_d = \frac{1}{H} \int^{H₁}_{H₀} \left( b₁ \left( 1 - e^{-b₂x} \right) - b₁ \left( 1 - e^{-b₂x} \right) \right) dx \] (3)

where \( b₂a \) is the coefficient of the first curve (control curve) and \( b₂b \) is the coefficient of the second curve (test curve). Control curve is the pressure-time relation in the absence of acetylcholine and test curve is the pressure-time relation in the presence of acetylcholine. Here \( b₁ \) is considered the same for both functions under the integral, which follows from the results (see below). The solution of the Eq. (3) is:

\[ P_d = \frac{b₁ \cdot b₂a - b₂b}{H \cdot b₂a \times b₂b} \] (4)

The calculated \( P_d \) is the area between the test and control curves and it is normalized to become a dimensionless quantity (Fig. 1B). 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 \( P_d \) indicates the shift of the test curve to the right and slower development of maximal pressure.

Further, we introduce the total \( P_d \) (\( TP_d \)) as the area under the test and/or control curves which can be calculated from the equation:

\[ TP_d = b₁ \left( x + \frac{e^{-b₂x}}{b₂} \right) - \frac{b₁}{b₂} \] (5)

where \( TP_d \) is total pressure dynamics and \( x = 5T \).

Statistical analysis

Data are presented as means ± S.E.M. (standard error of the mean). Comparison between groups was analyzed by one-way analysis of variance followed by a Tukey’s post hoc test.
(multiple comparison procedure). Also, data were analyzed using Student's $t$-test, where $p < 0.05$ was considered as statistically significant.

Results

In Table 1 we present serum levels of sodium, potassium, blood urea and creatinine in C-group, GM-group and GMC-group of animals. Levels of blood urea and creatinine in GM-group were significantly elevated in comparison with the C-group and GMC-group. Serum potassium concentration in GM-group of animals was significantly decreased when compared to C-group and GMC-group. Concentration of sodium in serum of GM-group was lower than in C-group and GMC-group, but without statistical significance.

The experimental pressure-time curves were analyzed using a method described in the Materials and Methods section and the values of $5T$, $b_1$ and $b_2$ coefficients of the fitted experimental data (coefficient of correlation was 0.979 ± 0.0048) in the control and experimental groups are shown in Table 2. Our data indicate that coefficient $b_2$ in GM-group is significantly higher when compared to C-group and GMC-group. On the other hand, the $5T$ value in GM-group is significantly lower in comparison with the C-group and GMC-group.

To describe the pressure dynamic properties of the blood vessels, the parameters $P_d$ and $TP_d$ were calculated (see Eqs. (4) and (5)) as shown in Table 3. Our data indicate that the $P_d$ as well as $TP_d$ coefficient in GM-group is significantly lower when compared to C-group and GMC-group. There is no difference of these coefficients in GMC-group in comparison with C-group.

Discussion

In this paper we use a new approach (Rosic et al. 2008) to $ex$ $vivo$ blood vessel 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. The end points of blood vessel response to some drugs, for instance, 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. Although we may know the effects of some drug to blood vessel smooth muscle, manifested through constriction or relaxation, we cannot precisely predict the time history of relevant biomechanical variables in between alternate steady states. In order to elucidate the mentioned phenomena, we introduced a new experimental design. This design is created to measure the changes in pressure when an abrupt change of pressure occurs at the vessel outlet, by applying various hydrostatic levels, as step functions. We evaluate the time history of the pressure changes in-between alternate steady states, represented by the pressure-time curves. Further, we describe the dynamic behavior of the vessel, by proposing a mathematical relation in which the parameters are evaluated through the pressure-time curves relationships with the

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Values are represented as mean ± S.E.M.* significantly different values.

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experimental data. In the simple exponential mathematical function, the two coefficients have physical meaning: the first coefficient, $b_1$, numerically describes the maximal developed pressure, i.e. the pressure corresponding to the alternate steady state; while the second coefficient, $b_2$, is relevant for the description of the vessel transition response between alternate steady states. Also, in order to define dynamic behavior of the blood vessel in a quantitative manner, we introduced two parameters: $P_d$ and $TP_d$ (Eqs. (4) and (5)).

In our previous work (Rosic et al. 2008) we demonstrated that these coefficients are very sensitive parameters to the conditions of the blood vessel dynamics.

Levels of blood urea, creatinine, potassium and sodium in GM-group, confirmed the well known pattern of gentamicin nephrotoxicity. Our results show that levels of blood urea and creatinine in GM-group were significantly elevated in comparison with the GCM-group. Also, serum potassium concentration in GM-group of animals was significantly decreased when compared to GCM-group. These findings are in accordance with previous reports (Kadkhodaee et al. 2005, 2007) confirming protective effects of antioxidants (including vitamin C) in gentamicin-induced nephrotoxicity.

Our results show that, in control and experimental groups of animals, there is no difference in $b_1$ coefficient. This means that the maximal pressure is the same in all groups of animals. The maximal pressure under the constant flow conditions only depends on the hydrostatic level in our experimental design, and thus the alternate steady state could not be affected. In the contrary, the $b_2$ coefficient in GM-group is significantly higher when compared to C-group and GCM-group, indicating alteration in dynamic response of blood vessels during the change of pressure in gentamicin-treated animals. The time interval within which the maximal pressure (alternate steady state) is developed decreases from $8.07\, s$ in C-group to $5.64\, s$ in GM-group, showing a faster pressure change, back to the control values. Calculated values of $TP_d$ coefficients which quantitatively describe the pressure dynamic properties, confirm the alteration in dynamic response of blood vessels during the change of pressure in gentamicin-treated animal and beneficial effects of vitamin C administration.

$P_d$ in all three groups of animals has a negative values indicating the shift of the exponential curves to the right in the presence of acetylcholine. This means that acetylcholine-induced vasodilatation leads to slower development of maximal pressure in all investigated animals. Our data indicate that the $P_d$ coefficient in GM-group is significantly lower when compared to C-group suggesting reduced vasodilatory response in gentamicin-treated animals. This findings are in accordance with recent investigation of coronary vessel reactivity in ARF (Kingma et al. 2006). The $P_d$ value in GM-group is significantly lower in comparison with the GCM-group, indicating vitamin C-induced restoration of vasodilatatory response back to the control values.

As mentioned in Introduction section, the molecular mechanisms underlying gentamicin-induced renal failure include increased free radicals production (Kays et al. 1991; Ben Ismail et al. 1994; Ata Secilmis et al. 2005). On the other hand, free radicals are also, involved in impaired vascular function in ARF, partly by promoting AOPP as mediators of oxidative stress (Shuangxu et al. 2005). From this point of view, antioxidants (including vitamin C) may have both, protective effects on gentamicin-induced nephrotoxicity (Kadkhodaee et al. 2005, 2007), as well as, direct protective effects on impaired vascular function in ARF. In addition, vitamin C-induced decreased level of blood urea in ARF may lead to lower production of cLDL and consequent prevention of vascular impairment.

References


