Nitric oxide and peroxynitrite balance in the cardiovascular system: a nanomedical approach

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Abstract

A small deviation from the physiological NO concentration may have a significant effect on the function of the cardiovascular system. A deficiency in NO production and concomitant increase in cytotoxic peroxynitrite ($ONOO^-$) has been associated with dysfunctional endothelium. Low level of bioavailable NO and high level of $ONOO^-$ are features of several vascular diseases including hypertension, arteriosclerosis, stroke and myocardial infarction. This study used a nanotechnological and nanomedical approach (nanosensors) to measure the release of NO and $ONOO^-$ from a single dysfunctional endothelial cell (HUVECs, aortic, cardiac, and endothelial cells). A balance of K=[NO]/[$ONOO^-$] was used as a criterion of endothelial function or dysfunction. Normal HUVECs produced high NO (480±35 nmol/L), low $ONOO^-$ (70±4 nmol/L) and a [NO]/[$ONOO^-$] balance was at the level of K=3.5. In the presence of ox-LDL, this balance decreased to K=0.32±0.02. A significant decrease in [NO]/[$ONOO^-$] was observed in hypertensive (SHR) rats (K=1.5±0.1) as compared to normotensive (WKY) rats (K=2.8±0.3). The K balance can be positively shifted by L-arginine or superoxide dismutase. In the failing heart of SHR, K value decreased to 0.38±0.04 from 2.2±0.2 for sham-operated rats. Treatment for 4 weeks with simvastatin (2.5 mg/kg/day) partially but significantly restored the [NO]/[$ONOO^-$] balance in SHR with a failing heart. Therefore, simvastatin-induced shift of [NO]/[$ONOO^-$] balance may contribute to the clinical usefulness of simvastatin in the treatment of the failing heart.

Key words: dysfunctional endothelium, nitric oxide, peroxynitrite, statins, heart, vasculature.

Streszczenie

Niewielkie odchylenia w stężeniu fizjologicznego NO mogą mieć istotny wpływ na funkcję układu krążenia. Upośledzenie produkcji NO ze współtowarzyszącym wzrostem cytotoksycznego nadtlenoazotynu ($ONOO^-$) jest związane z dysfunkcją endotelium. Niski poziom biodostępnego NO i wysoki $ONOO^-$ występuje w chorobach układu krążenia, takich jak: nadciśnienie, miażdżyca, udar mózgu i zawał serca.

Do pomiaru uwalniania NO i $ONOO^-$ z pojedynczej uszkodzonej komórki śródbłonka (HUVEC, aorty, serca i komórek śródbłonka) wykorzystano nanosensor wytworzony za pomocą nanotechnologii. Jako kryterium dysfunkcji śródbłonka przyjęto poziom równowagi pomiędzy stężeniem NO i $ONOO^-$ (K=NO/$ONOO^-$). W otoczeniu prawidłowych komórek HUVEC występowały wysokie stężenia NO (480±35 nmol/L), a niskie $ONOO^-$ (70±4 nmol/l), natomiast stała równowagi [NO]/[$ONOO^-$] wynosiła K=3,5. Przy obecności ox-LDL, stała równowagi przesuwała się do poziomu K=0,32±0,02. Istotny spadek stosunku [NO]/[$ONOO^-$] obserwowano u szczurów z nadciśnieniem (SHR) (K=1,5±0,1) w porównaniu ze szczurami normotensyjnymi (WKY) (K=2,8±0,3). L-arginina i dysmutaza nadtlenkowa powodowały przesunięcie stałej równowagi w kierunku wyższych wartości. Stosowanie simwastatyny przez 4 tyg. (w dawce 2,5 mg/kg/dobę) u SHR z niewydolnym sercem spowodowało częściowe, ale istotne przywrócenie równowagi [NO]/[$ONOO^-$]. Tak więc simwastatyna indukuje przesunięcie równowagi [NO]/[$ONOO^-$] i może znaleźć zastosowanie w leczeniu niewydolności serca.

Słowa kluczowe: dysfunkcja środbłonka, tlenek azotu, nadtlenoazotyn, statyny, serce, układ naczyniowy.

Introduction

The vascular endothelium is a dynamic endocrine organ that regulates contractile, secretory, and mitogenic activities in the vessel wall and the hemostatic process within the vascular lumen [1, 2]. Endothelial nitric oxide is involved in the normal regulation of vasomotor tone and blood pressure by stimulating smooth muscle relaxation, inhibiting platelet aggregation and adhesion to the endothelium and preventing smooth muscle proliferation. A mechanism of NO signalling involves activation of soluble quanylyl cyclase and increased intracellular cyclic quanosine monophosphate (cGMP) in vascular smooth muscle and platelets.

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Nitric oxide is produced in the endothelium from L-arginine and molecular oxygen and this synthesis is catalyzed by a constitutive endothelial nitric oxide synthase (eNOS) [3, 4]. The generation of NO in cardiovasculature can be stimulated by mechanical stimuli (flow induced shear stress) and chemical stimuli (acetylcholine, ATP, ADP, bradykinin, serotonin, thrombin and others) [5, 7]. A vascular endothelium under conditions of blood flow is covered by a thin film of NO and the concentration of NO in this film can be in low nanomolar range (1 – 60 nmol/L). The concentration of NO depends on the type of artery and the type of flow (laminar vs. turbulent flow). The highest concentration has been observed in the heart [6].

A small deviation from the physiological NO concentration may have a significant effect on the function of the cardiovascular system [8]. A deficiency in NO production has been associated with dysfunction of the endothelium. Dysfunctional endothelium and diminished NO production are features of several vascular diseases including hypertension, arteriosclerosis, ischemia/reperfusion, stroke and myocardial infarction. Overproduction of NO is a main feature of septic shock and hypotension. A dysfunctional endothelium can generate higher amounts of superoxide (O_2^-) than a normal endothelium. Subsaturating concentrations of tetrahydrobiopterin (BH_4) and/or L-arginine promote eNOS uncoupling in dysfunctional endothelium and one electron reduction of O_2 to O_2^-. NO and O_2^- can react in a rapid diffusion controlled reaction to form peroxynitrite (ONOO-), a highly cytotoxic compound [9]. The probability of a reaction between O_2^- and NO is very high because both these molecules can be produced in close proximity by the same enzyme, eNOS. At a low concentration, $ONOO^-$ can undergo rapid isomerization to form nitrate (NO_3^-). However, at high concentration, the gradient of $ONOO^-$ established between the membrane of the endothelial cell and cytoplasm, and the membrane of the endothelial cell and blood, facilitates its diffusion. During the diffusion process the $ONOO^-$ molecule undergoes homolytic or heterolytic cleavage. The homolytic cleavage leads to the production of hydroxyl radicals (OH·) and nitrogen dioxide radicals ($NO_2^{\cdot}$). Both of these radicals are very strong oxidants. The hetorolytic cleavage of $ONOO^-$ produces another strong oxidant, nitronium ion (NO_2^+). O_2^-, $ONOO^-$, NO_2, OH· and NO_2^+, are the major components of oxidative and nitroxidative stress generated by the dysfunctional endothelium. Therefore, a balance between bioavailable NO and oxidative/nitroxidative stress (reflected by the concentration of $ONOO^-$) can be used to diagnose the function or dysfunction of endothelium in the cardiovascular system [2, 8]. This work describes an application of nanosensing/nanotechnological/nanomedical techniques for *in situ* monitoring of the two molecules, NO and $ONOO^-$, which are crucial in the function and/or dysfunction of the cardiovascular system.

Materials and methods

Cell culture

Human umbilical vascular endothelial cells (HUVECs) were isolated from the umbilical vein with 0.5 mg/mi collagenase and 0.25% trypsin digestion. The HUVEC culture was incubated in 95% air, 5% CO_2 at 37ºC [10]. The confluent cells (4-5x10^5 cells/35-mm dish) were placed with a minimum essential medium containing 5% human lipoprotein-depleted serum. For experimentation, the cells were placed on glass cover-ups and grown to confluence.

Animals

Nine-month-old male and female normotensive (WKY) and hypertensive (SHR) rats from Harlan, Indianapolis, IN (USA) weighing 330-360 g (male) and 200-230 g (female), were used in this study. All experiments were performed in accordance with the protocols approved by the committee of the Institutional Animal Welfare and Safety Compliance Office.

Preparation of the tandem sensors for NO and $ONOO^-$ detection

Concurrent measurements of NO and $ONOO^-$ were performed with electrochemical nanosensors [10-14] combined into one working unit with a total diameter of 2-3 µm [10]. Their design was based on previously developed and well-characterized chemically modified carbon-fiber technology. Each of the sensors was made by depositing a sensing material on the tip of a carbon fiber (length 4-5 µm; diameter ~200 nm). The fibers were sealed with a nonconductive expoxy and electrically connected to copper wires with conductive silver epoxy. We used conductive film of polymeric nickel(II)tetrakis(3-methoxy, 4-hyroxyphenyl)porphyrin for the NO sensor, and polymeric film of Mn(III)-[2.2]paracyclophenylporphrin for the $ONOO^-$ sensor [15].

Measurement of NO and $ONOO^-$ in a single endothelial cell

A module of NO/$ONOO^-$ nanosensors (working electrodes) with platinum wire (0.1 mm) counter electrode and saturated calomel referenced electrode was applied. Differential pulse voltammetry and amperometry were performed with a computer-based Gamry VFP600 multichannel potentiostat. Differential pulse voltammetry was used to measure basal NO and $ONOO^-$ concentrations and amperometry was used to measure changes in NO and $ONOO^-$ concentrations from the basal level with time. The differential pulse voltammetry current at the peak potential characteristic for NO (0.65 V) oxidation and $ONOO^-$ (-45 V) reduction was directly proportional to the local concentrations of these compounds in the immediate vicinity of the sensor. Linear calibration curves (current vs. concentration) were constructed for each sensor from 10 nmol/L to 2 µmol/L before and after measurements with aliquots of NO and $ONOO^-$ standard solutions, respectively. The detection limit of the sensors was 10^{-9} mol/L. The concentration of each analyte was calculated from the peak current of an amperogram and a standard calibration curve. At a constant distance of the sensors from the surface of the endothelial cell, reproducibility of measurements is relatively high (5% to 12%).

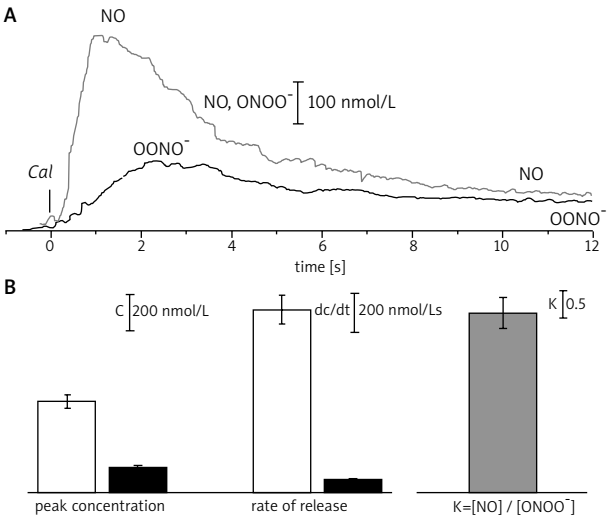

Fig. 1. Typical amperograms showing changes of nitric oxide and peroxynitrite concentration produced by a single HUVEC (A); NO and $ONOO^-$ release was stimulated by calcium ionophore A23187 (1 µmol/L); maximal concentration rate of release dc/dt and K [NO]/[$ONOO^-$] balance for NO (open bars) and $ONOO^-$ (solid bars produced by a single HUVEC (B); (n=5)

The position of nanosensors (*x, y* and *z* coordinates) versus the endothelial cell was established with the help of a computer-controlled micromanipulator. To establish a constant distance from cells, the module of sensors was lowered until it reached the surface of the cell membrane (a small piezoelectric signal, 0.1 to 0.2 pA, of 1- to 3-ms duration was observed at this point). After that, the sensors were slowly raised 4±1 µm (*z* coordinates) from the surface of a cell culture. The sensors were then moved horizontally (*x, y* coordinates) and positioned above a surface of randomly chosen single endothelial cells (at least 10 mm apart from the endothelium cell which was used to establish the *z* coordinate). The eNOS agonist calcium ionophore A23187 (CaI) was then injected using a nanoinjector that was also positioned by a computer-controlled micromanipulator.

Measurement of NO and $ONOO^-$ in a beating heart

Whistar-Kyoto rats were anesthetized (100 mg/kg ketamine and 10 mg/kg xylazine), incubated, and ventilated with room air using a Harvard small animal ventilator (tidal volume of 2.5 mL and rate of 100 breaths/min. After a median sternotomy was performed, cardiac NO concentration was measured as follows: to implant the nanosensors, ventricular tissue was pierced with a standard angiocatheter needle, 0.8 mm diameter (clad with catheter and with two 100-µm ventilation holes near the tip) [6]. The catheter/needle unit was advanced to a desired place in the heart. The position of the catheter was secured and the placement needle was removed and quickly replaced with a tandem of nanosensors mounted in a truncated needle. Confirmation of myocardial localization of the active tip of the sensor was by postmortem sectioning of the heart.

Preparation of rats with chronic heart failure

Myocardial infarction (MI) was induced in SHR according to the method described previously [15]. The rats were anaesthetized with ketamine/xylazine (35/2 mg/kg) intraperitoneal. Lidocaine (2 mg/kg) intra-muscular was given to avoid ventricular arrhythmias after induction of MI. Following incubation, ventilation and performance of left thoracotomy, the left coronary was ligated 2 mm distal to the aortic origin. One week after surgery, the animals with MI were randomly divided into two groups, which were treated with either placebo or simvastatin. The rats received simvastatin 2.5 mg/kg/day orally by gastric gauge for four weeks. Five weeks after surgery, the animals were anaesthetized and subsequently heparinised (heparin 500 I.U./100 gl). After that, the hearts were rapidly removed for determination of NO and ONOO-.

Calculations and statistical analysis

Statistical analysis was done using Student's *t* test. For multiple comparisons, results were analyzed by ANOVA followed by Bonferroni and Dunn's correction. Data are presented as the mean ± SEM. Mean values were considered significantly different at $P<0.05$.

Results

Kinetics of NO and $ONOO^-$ release from a single HUVEC

The profiles (amperograms) of NO and $ONOO^-$ concentrations were recorded simultaneously by a tandem of nanosensors placed at 4±1 µm from the surface of the single endothelial cell (Fig. 1). 280±40 ms after stimulation of eNOS with a receptor-independent agonist (calcium ionophore, A23187 1 µmol/L), NO release was observed. A maximal NO concentration of 480±35 nmol/L was reached after 500±40 ms. The rate of NO production was 960±75 nmol/L. The rate of A23187 stimulated $ONOO^-$ was 70±4 nmol/Ls and maximum concentration of 140±8 nmol/L was reached after 2.0±0.3 s. The ratio K of NO concentration to $ONOO^-$ concentration was 3.4±0.3 (Fig. 1B).

After incubation of HUVECs with ox-LDL (50 mg chol/dl) the kinetics of NO and $ONOO^-$ release as well as its concentration balance changed significantly (Fig. 2). The maximal concentration of bioavailable NO decreased to 120±5 nmol/L, which is about 75% lower than the control. The rate of NO release also decreased threefold. The decrease in NO concentration was parallel with an increase in $ONOO^-$. Level of $ONOO^-$ increased from 70±4 nmol/L in controls to 760±30 nmol/L in ox-LDL treated cells. The K ratio reflecting NO/$ONOO^-$ balance dramatically decreased to 0.32±0.02 for ox-LDL treated cells.

There was a significant difference between NO and $ONOO^-$ concentration produced in aortic endothelium of normotensive (WKY) rats and hypertensive (SHR) rats (Fig. 3). A single aortic endothelial cell of WKY rats produced a maximal 505±30 nmol/L of bioavailable NO and 180±15 nmol/L

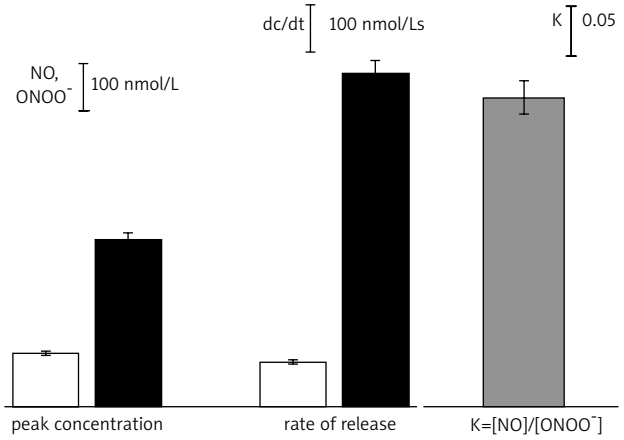

Fig. 2. Peak concentration, maximal rate of release, and K = [NO]/[$ONOO^-$] balance at NO and (open bars) $ONOO^-$ (solid bars) produced by a single HUVEC in the presence of ox-LDL. HUVECs were incubated with ox-LDL (60 mg/ml) for 12 hrs; (n=5)

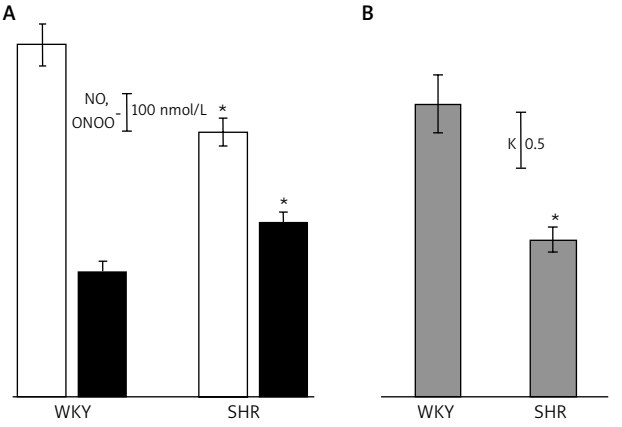

Fig. 3. Maximal concentrations (A) and K=[NO]/[$ONOO^-$] balance (B) for NO (open bars) and $ONOO^-$ (solid bars) produced by a single aortic endothelial cell of WKY and SHR rats SHR vs. WKY, p<.001, n=5

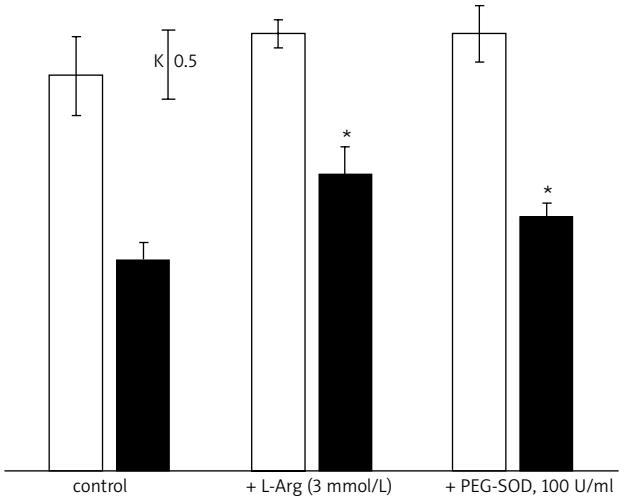

Fig. 4. Effect of elevated concentrations of L-arginine and PEG-SOD on NO/$ONOO^-$ balance of aortic endothelial cells of WKY (open bars) and SHR (solid bars) rats. * vs. control, p<.01, n=5

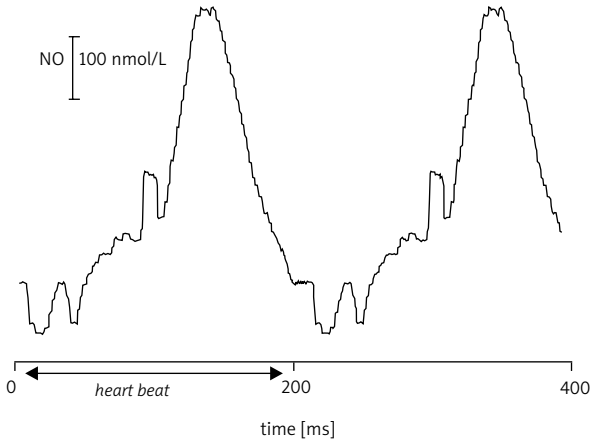

Fig. 5. Amperograms showing the changes in NO concentration in the beating heart of an SHR rat. NO concentrations were measured with catheter protected nanosensor implanted in the left ventricular wall

of $ONOO^-$ (Fig. 3). The production of NO decreased and $ONOO^-$ increased in the endothelium of SHR rats: NO maximal concentration in SHR rats was lower (380±20 nmol/L) while $ONOO^-$ concentration was higher (250±15 nmol/L) than the concentrations observed for WKY rats. The ratio K decreased twofold in SHR as compared to WKY rats (Fig. 3B). At elevated concentration of L-arginine (3 mmol/L) or in the presence of membrane permeable PEG-SOD (100 U/ml) the bioavailable NO concentration was partially restored and $ONOO^-$ concentration decreased. Therefore, a significant overall improvement (increase) in K value was observed (Fig. 4).

Nitric oxide and peroxynitrite in the heart

Nanosensors placed in the mid-ventricular myocardium of a WKY rat recorded rapid changes in NO concentration (Fig. 5). In the heart of the rat, each cardiac cycle began and ended with intercycle concentration of 210±20 nmol/L. NO in the heart is produced during systole and diastole. Early diastolic filling was accompanied by a brisk increase in NO with a peak diastolic NO concentration of 750±40 nmol/L. After this peak, there was a sharp decay to the intercycle NO concentration. In a set of separate experiments a chronic effect of simvastatin on endothelial NO and $ONOO^-$ production in the SHR rat after myocardial infarction (MI) was observed. Heart failure was induced by permanent occlusion on the left coronary artery. Calcium ionophore- stimulated NO release from the cardiac endothelium of SHR was reduced from 520±30 sham-operated to 240±20 nmol/L in the SHR with chronic heart failure animals (Fig. 6a). Also, the chronic heart failure led to a dynamic increase of CaI stimulated generation of $ONOO^-$.

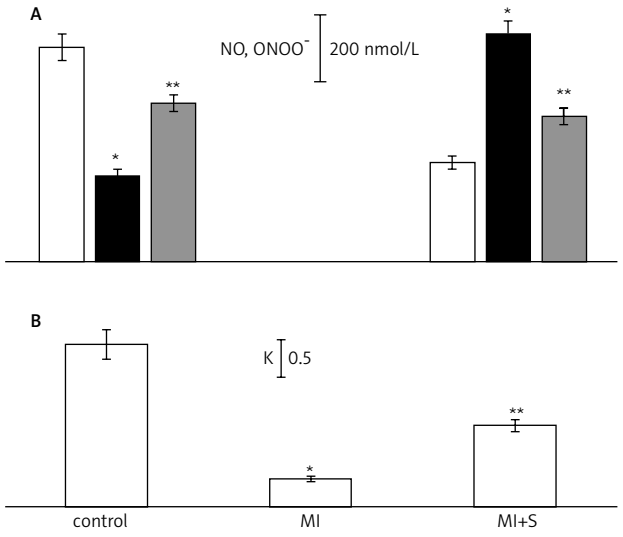

Fig. 6. Maximal concentration of NO and $ONOO^-$ (**A**) and K=[NO]/[$ONOO^-$] balance (**B**) in left ventricle wall of SHR, five weeks after myocardial infarction (MI). Sham (placebo-treated sham-operated rats (open bars); rats with myocardial infarction, placebo treated (solid bars); rats with myocardial infarction treated with simvastatin (MI + S) for 4 weeks (gray bars). NO and $ONOO^-$ release was measured *ex vivo* after stimulation with calcium ionophore (CaI, A23187, 1 µmol/L). *MI vs. placebo-treated, sham-operated, p<.001 (n=5); **MI + S vs. MI, p<.001 (n=5)

Concentration of $ONOO^-$ increased from 80±10 nmol/L in sham-operated animals to 240±20 nmol/L in animals with a failing heart. Four weeks of treatment of infarcted animals with simvastatin (2.5 mg/kg/day) significantly enhanced NO production. Also, the treatment with simvastatin significantly reduced the concentration of $ONOO^-$ in these animals. The ratio of NO/$ONOO^-$ concentrations was 0.38±0.04 in SHR with failing heart, which is about sixfold lower than in sham-operated animals (Fig. 6b). However, the treatment with simvastatin partially but significantly restored NO/$ONOO^-$ ratio to a level of 1.10±0.08, which is only two times lower than the K value measured for sham-operated SHR rats.

Discussion

This study provides direct evidence that a balance between the bioavailable NO and cytotoxic $ONOO^-$ can be changed dramatically in dysfunctional endothelium. The nanotechnological/nanomedical approach employed in this study allowed *in situ* monitoring of both NO and $ONOO^-$simultaneously with a time resolution better than a millisecond at a nanomolar level of concentrations. This study showed also that NO/$ONOO^-$ balance can be shifted positively and the function of endothelium can be restored after treatment with L-arginine, SOD and more significantly after treatment with simvastatin.

Treatment of the ischaemic heart with simvastatin significantly reduced nitroxidative stress ($ONOO^-$) and restored bioavailable NO, bringing the ratio of concentration of these two molecules in the endothelium of SHR rats with failing hearts to a similar level. The data presented here strongly indicate that a high NO/$ONOO^-$ balance is beneficial for optimal function of the endothelium. In normal endothelium, the fast kinetics of NO release promotes the build-up of a significant gradient of NO concentration between the endothelial cell membrane/cytostolic interface and the endothelial cell membrane/blood interface. This high concentration gradient facilitates fast diffusion and propagation of NO and efficient regulation of vascular tone.

Calcium flux/calmodulin stimulated NO release by eNOS in a normal endothelial cell reached the maximal concentration after less than 500 ms. The duration of a "puff" of nitric oxide produced by the endothelium is limited by the production of O_2^- by eNOS and other sources. O_2^- produced by eNOS is a main scavenger of NO due to the close proximity of the release of these two molecules this same enzyme [9]. The scavenging of NO by O_2^- which is also produced by eNOS is fast (9.6×10^9 mol/s). This scavenging process regulates acute overproduction of NO while phosphorylation of eNOS regulates chronic NO overproduction. The product of diffusion controlled reaction between NO and O_2^- is peroxynitrite. Peroxynitrite with $t_{1/2}$, <1s, is less stable than NO ($t_{1/2}$, <3s) in a biological milieu. When generated at low concentration, propagation of $ONOO^-$ by diffusion is very slow due to a low concentration gradient. Under slower diffusion conditions, $ONOO^-$ isomerizes to produce harmless nitrite. However, at higher concentrations, a significant portion of protonated $ONOO^-$ can diffuse through the cell membrane.

During the propagation process the ONOOH molecule can undergo cleavage to several highly oxidative species including NO_2^+, $OH^\cdot$ and $NO_2^\cdot$. These species are a main component of oxidative/nitroxidative stress and can initiate a cascade of events leading to necrosis, apoptosis, nitration, enzyme deactivation, vasoconstriction, edema formation and others. Therefore, upregulation of $ONOO^-$ production by dysfunctional endothelium can have deleterious effect on endothelial function (contraction of vascular wall, induction of platelet/platelet, platelete/leukocyte aggregation, adhesion) and can accelerate plaque formation in the vasculature [16].

These studies provide answers to several questions regarding what concentration levels of NO and $ONOO^-$ are generated by normal and dysfunction endothelium. Human endothelial cells exposed to ox-LDL showed a dramatic increase in $ONOO^-$ and diminished levels of bioavailable NO. Thus, the overall NO/$ONOO^-$ balance was unfavorably shifted from about 3.5 to about 0.32±0.02. A significant shift of NO/$ONOO^-$ balance was observed also in the endothelium of hypertensive rats. The NO/$ONOO^-$ balance in dysfunctional endothelium can be partially restored by supplementation of L-arginine (substrate of NO) or antioxidants (superoxide dismutase). L-arginine treatment reduces generation of O_2^- in dysfunctional endothelium and is relatively effective in reducing $ONOO^-$ level and in-

creasing NO. Elevation of L-arginine from its physiologic level of 0.8 mmol/L to about 2-3 mmol/L can stabilize the dimeric form of eNOS and prevent generation of high concentrations of O_2^-.

Inhibition of the release of O_2^- is more effective than the scavenging of O_2^- by antioxidants and/or superoxide dismutase [9]. The kinetics of O_2^- dismutation ($K \approx 10^9$ mol/s) are much slower than the kinetics of NO reaction with O_2^- (6×10^9 mol/s). Therefore, the scavenging/dismutation of O_2^- would require very high concentrations of antioxidants or superoxide dismutase in order to compete effectively with the fast NO, O_2^- reaction. Treatment with simvastatin appears to be highly beneficial and effective for the restoration of eNOS function and also for the significant positive shift of NO/$ONOO^-$ balance. We have reported previously a pleiotropic effect of cerivastatin on endothelial NO release from isolated endothelial cells [15]. Here we demonstrate *in vivo* a beneficial effect of simvastatin on the restoration of NO/$ONOO^-$ balance in the failing heart.

Treatment with simvastatin significantly enhanced the NO bioavailability of the cardiac endothelium from SHR with severe chronic heart failure induced by permanent left coronary artery ligation. This effect of statin is probably partially due to the enhanced endothelial NO production along with reduced O_2^- by calcium-dependent eNOS. In the heart NO acts directly on myocytes via an increase in cGMP to facilitate relaxation (positive lusitropy) and to mediate Ach-stimulated decreases in contractibility (negative inotropy) [6, 17, 18]. A similar effect of increased NO concentration in chronic heart failure of SHR previously treated with the angiotension-converting enzyme inhibitor ramipril was observed [14].

Conclusions

Production of bioavailable NO by dysfunctional endothelium is diminished while the generation of cytotoxic $ONOO^-$ can be significantly enhanced. Therefore, the overall balance of NO/$ONOO^-$ is shifted to a very low level. At this low level of NO/$ONOO^-$ diffusion and signalling of NO are hindered and the vital role of this molecule in the regulation of vascular tone is diminished. In addition, at high concentration $ONOO^-$ can generate a cascade of cytotoxic radicals and induce oxidative damage to components of the cardiovasculature: platelets, leukocytes, endothelial cells and enzymes. Pharmacological intervention with simvastatin restored NO/$ONOO^-$ balance to a higher level, increased bioavailability of endothelium-derived NO and decreased the cytotoxic effect of $ONOO^-$. Both of these favourable effects may contribute to the clinical usefulness of simvastatin in the treatment of heart failure.

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