Dynamics of Blood Nitric Oxide Level During Nitric Oxide Inhalation

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ABSTRACT. Nitric oxide (NO) which is produced from L-arginine by nitric oxide synthase (NOS) is a key molecule in regulation of hemodynamics and organ blood flow. Inhalation of NO has been applied in various diseases, such as pulmonary hypertension and prevention of chronic lung disease in the newborn, congenital heart disease, lung transplantation, acute respiratory distress syndrome, and chronic obstructive pulmonary disease. Although inhalation of low levels of NO appears to be safe and cause a rapid increase in lung blood flow without causing systemic hypotension, influence of NO inhalation to the kinetics of NO in the systemic circulation has not been clarified. Therefore, we have measured the plasma NO concentration in the porcine aorta and femoral artery by employing the NO-selective electrodes during NO inhalation. We located the NO sensors through a 5-Fr sheath in the aortic arch and left femoral artery. NO was administered with a NO inhalation device from a respirator. While inhaling NO (1 and 10 ppm), plasma NO concentration increased both in the aortic arch and in the left femoral artery. The inhalation of NO influences the kinetics of NO in blood beyond the pulmonary circulation.

Key words ① **Nitric Oxide** ② **NO inhalation** ③ **NO sensor** ④ **direct measurement of NO** ⑤ **NO**

Nitric oxide (NO), which is produced from L-arginine by nitric oxide synthase (NOS), is a key molecule in the regulation of hemodynamics and organ blood flow. NO produced by endothelial cells causes vascular smooth muscle to relax and inhibits the aggregation of blood platelets and the adhesion of leukocytes, thus maintaining organ blood flow and vascular integrity[1),2)]. Since the therapeutic potential of inhaled NO as a selective pulmonary vasodilator was firest suggested in patients with pulmonary hypertension[3)], it's vasodilative action has been applied in the treatment of various diseases, such as for pulmonary hypertension and the prevention of chronic lung disease in the newborn[4)], congenital heart disease, lung transplantation, acute respiratory distress syndrome, and chronic obstructive pulmonary disease[5)~12)]. Inhalation of low levels of NO appears to be safe and causes a rapid increase in lung blood flow without causing systemic hypotension. The underlying hypothesis of selective pulmonary vasodilation is based on the rapid scavenging of NO by hemoglobin in the pulmonary circulation. However, recently proposed possible mechanisms that conserve and stabilize NO might allow inhaled or injected NO to have cardiovascular effects far from the site of delivery. In fact, in our recent study, we found that NO concentration increased in the canine aorta

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immediately after the injection of acetylcholine into the left ventricle[13),14)]. Accordingly, we wondered whether inhalation of NO might increase the plasma NO concentration in the systemic circulation. Herein we present the results of direct measurement of the plasma NO concentration in the porcine aorta and femoral artery by NO-selective electrode during NO inhalation.

MATERIALS AND METHODS

NO sensors

Our NO sensors were 700 μm in diameter and 150 mm in length (amiNO-700, Innovative Instruments, Inc., Tampa, FL, USA), and was connected to the inNO-T (Innovative Instruments), an NO-measuring system including a current monitor and data acquisition software with a temperature compensation function that automatically compensates for sample temperature fluctuation. The electric signal due to the oxidation of NO at the sensing element was recorded, and converted to the NO concentration. (Fig 1)

Calibration of the NO sensors

The detection tip of each NO sensor was immersed in a well-stirred saline solution (50 ml). NO-saturated pure water was prepared by bubbling pure NO gas in oxygen-free pure water for 15 min. Using a gas-tight syringe, 5 μl of the solution was injected into the saline solution (final NO concentration: 190 nmol/l).

ANIMAL PREPARATION

Ten-week-old male pigs (n=4) were sedated with intramuscular ketamine (10 mg/kg) before sevofluren (0.5 - 4%), fentanyl (0.3 mg) and pancronium bromide (4 mg) induction and endotracheal intubation. Their weights ranged from 28 to 32 kg. A temperature probe was inserted into the rectum, a pulse oximeter probe was placed on the tongue, and electrocardiogram leads were affixed to the chest. While the animals received continuous sevofluren anesthesia, a carotid artery cutdown was performed.

A carotid artery catheter was placed in the artery for continuous systemic blood pressure monitoring and arterial blood gas analysis. Cutdown of the contralateral femoral vein allowed for large-caliber intravenous access and the infusion of maintenance fluid (normal saline, 4 ml/kg/h). The animals were transferred to continuous volume-controlled mandatory ventilation. The ventilator parameters were adjusted to achieve $PaCO_2$ of 35 to 45 mmHg. At the end of the experiment, the animals were sacrificed with KCl (40 mEq/kg). These experiments were approved by the Animal Research Committee of Kawasaki Medical School (NO.

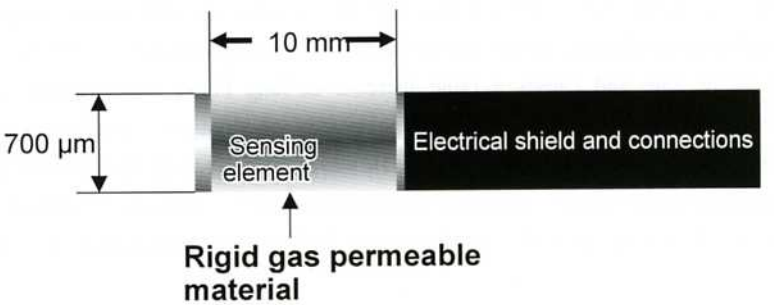

Fig. 1. NO sensor

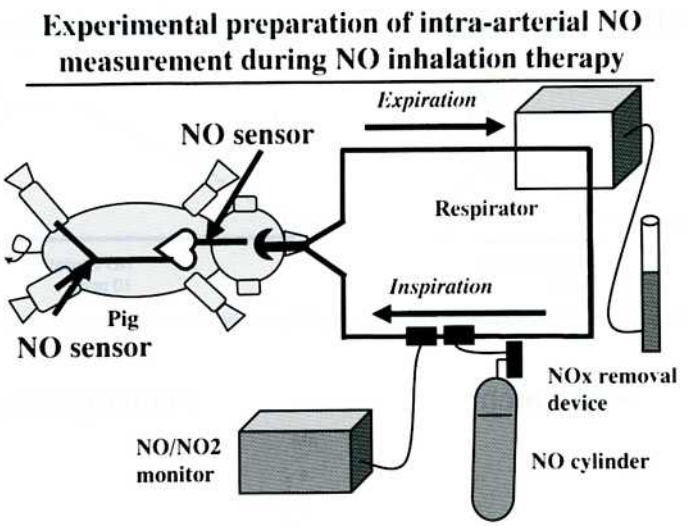

Fig. 2. Locations of two NO sensors (aortic arch and femoral artery)

04-118. 2004) and conducted according to the "Guide for the Care and Use of Laboratory Animals" of Kawasaki Medical School and adhered to the National Institutes of Health Guidelines on the Care and Handling of Experimental Animals.

EXPERIMENTAL PREPARATION

We mounted the NO sensors in the aortic arch and the left femoral artery (Fig. 2). NO was administered with a NO inhalation device (Taiyo Toyo, NO/NO_2 GAS MONITOR, TMS-100, Sano CO., Ltd) from a respirator. A mass flow regulator in NO/N_2 balance gas (800 ppm NO), which is used clinically as a NO donor was employed, and the pigs inhaled NO gas through the respirator. To remove remaining NO in the exhaust gas, the gas was treated through the exhalation circuit mounted with a soda-lime absorber. The plasma NO concentration was measured while the pigs inhaled NO (1 and 10ppm).

RESULTS

Representative changes in the plasma NO concentration in the left femoral artery during inhalation of NO (1 and 10 ppm) are shown in Fig. 3. The plasma NO concentration increased both in the left femoral artery by inhalation of NO (1 ppm and 10ppm) was begun and reached a steady level (1.4 nM and 2 nM in the left femoral artery) in 30 min (Fig. 3). The plasma concentration of NO increased 6.1 nM in the aortic arch and 8.0 nM in the left femoral artery by inhaling NO of 10 ppm. (Fig.4) Such increases in NO concentration were observed in the all animals studied. The increase in plasma NO concentration during the inhalation of NO (1 to 10 ppm) ranged from 0.01 to 13.0 nM.

DISUCUSSION

To the best of our knowledge, the present study is the first direct measurement of the concentration of

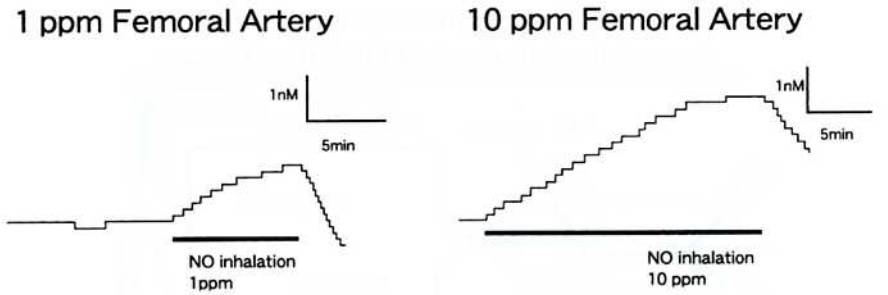

Fig. 3. Change in NO concentration during NO gas inhalation (1 ppm and 10 ppm)

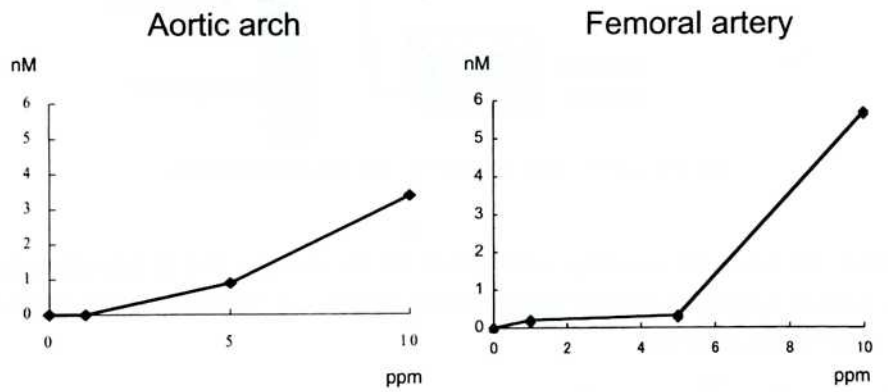

Fig. 4. Summanry of changes in plasma NO concentrations in the aortic arch and femoral artery (NO gas: 1 ppm and 10 ppm).

plasma NO in the porcine aorta and femoral artery during NO inhalation. The major new findings are i) concentration of plasma NO can be measured stably by the NO sensors, ii) inhalation of NO increases the plasma NO concentration in the aortic arch and even in the femoral artery at the therapeutic concentration of NO which does not cause significant systemic hypotension, and iii) the increase in the plasma NO concentration in the systemic arterial vessels seems to be dependent on the inhaled NO concentration.

Before these findings are discussed, some methodological problems must be addressed. Firstly, the evaluation of the plasma NO concentration during inhalation of NO depends on the validity of the NO-measuring system employing the NO selective microelectrode. In our previous in-vitro study, the NO sensor showed no noticeable change in the baseline current with and without fluid (blood) motion (data not shown)[13]. This NO sensor showed high specificity to NO. The validity of our in-vivo NO measuring system has previously been demonstrated extensively in the measurement of intra-aortic NO concentration following the infusion of acetylcholine into the left ventricle[14].

Secondly, because of the small number of experiments, we cannot conclusively report a relationship between plasma NO concentration and inhaled NO concentration. Nevertheless, increase in the plasma NO concentration with the inhalation of NO was consistently observed in all the animals studied. While the presented data suggest that the increase in plasma NO concentration depends on the concentration of inhaled NO (Fig. 4), further studies are needed to determine the relationship between the concentration of plasma NO and the concentration of inhaled NO. Future studies must also determine the possible influences of the plasma NO concentration during NO inhalation on systemic hemodynamics and organ blood flow and on the possible

remote vascular effects of NO, including the maintenance of vascular integrity.

CONCLUSION

The plasma NO concentration can be stably evaluated during NO inhalation. A therapeutic concentration of NO for inhalation increases the plasma NO concentration in the aortic arch and in the femoral artery. The increase in the plasma NO concentration with NO inhalation may contribute to systemic hemodynamics and organ blood flow and the maintenance of vascular integrity.

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