

# What Characterizes Endothelial Dysfunction in Preeclampsia? -The Action of NO and the Production of Prostacyclin is Reduced, While EDHF is Preserved in Omental Resistance Arteries in Preeclamptic Women

Yoshikatsu Suzuki<sup>1\*</sup>, Tamao Yamamoto<sup>1</sup>, Yoshimasa Watanabe<sup>2</sup> and Takeo Itoh<sup>2</sup>

<sup>1</sup>Departments of Obstetrics and Gynecology and <sup>2</sup>Cellular and Molecular Pharmacology, Graduate School of Medical Sciences, Nagoya City University, Nagaya, Japan

**Abstract:** Vascular endothelial cells release vasorelaxing factors (endothelium-derived relaxing factor; EDRF), such as nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor (EDHF), and these play an important role in the regulation of vascular tone, vascular permeability and blood coagulation, thus helping to maintain circulatory homeostasis.

Preeclampsia is characterized by marked increases in peripheral vascular resistance and vascular permeability together with a disturbance of blood coagulation. It has been suggested that an abnormality in the role played by EDRF in resistance arteries may be involved in the pathogenesis and/or development of preeclampsia.

*In vitro* investigation of characteristic changes in preeclampsia using vascular strips of omental resistance artery obtained from preeclamptic women, revealed that; 1) the action of endothelial NO is reduced not due to decrease in the production of NO in the endothelium but rather to reduced action of guanosine-3',5'-cyclic monophosphate (cGMP; a second messenger of NO) itself and/or downstream of cGMP, 2) reduced production of prostacyclin in endothelium and, 3) reservation of EDHF action. Taken together, it was suggested that EDHF might compensate for both the reduced action of endothelial NO and the reduced production of prostacyclin in resistance arteries. In this review the new observation of functional changes in endothelium seen in preeclampsia is important information of obstetricians.

## INTRODUCTION

Normal pregnancy is associated with profound changes in the maternal cardiovascular system, which adapts to accommodate the demands of growing fetus. Both blood volume and cardiac output increase by 40-50% over non-pregnant levels during the first trimester. Despite this, there is a simultaneous, progressive fall in the arterial blood pressure, beginning in the first trimester, reaching a nadir in mid pregnancy, and returning to pre-pregnant values at term. This fall in blood pressure is mediated by a decrease in the total peripheral resistance. From multiple studies, pregnancy reduces total peripheral resistance due to the decreased response to vasospasmogenic agonists and the increased action of endothelium-derived relaxing factors (EDRF).

Preeclampsia is characterized by marked increases in peripheral vascular resistance during pregnancy. There are two explanations for the increase in vascular resistance in patients with preeclampsia; 1) an increase in contractile response to vasospasmogenic factors and 2) reduced action of the EDRF. However, the underlying mechanisms are poorly understood in preeclampsia.

## CONTRACTILE RESPONSE IN PREECLAMPSIA

Normally, pregnant women develop refractoriness to infused vasopressors [1]. Increased vascular reactivity to pressors in women with early preeclampsia has been

identified using norepinephrine, angiotensin II and vasopressin [2]. Furthermore, Gant *et al.* demonstrated that increased vascular sensitivity to angiotensin II preceded the onset of preeclampsia [3].

It has been found that the contractile response to both angiotensin II [4] and vasopressin [5] is up-regulated in omental resistance arteries in patients with preeclampsia, in comparison with the situation in normotensive pregnant women.

Recently, it was found that in omental arteries obtained from preeclampsia, a significant increase in heterodimerization occurs between the angiotensin II type1 receptor for the vasopressor angiotensin II and B<sub>2</sub> receptor for vasodepressor bradykinin, indicating that the heterodimerization might cause the increased response to angiotensin II [6].

In the omental resistance arteries obtained from patients with preeclampsia compared with those in normotensive pregnant women, the action of STA<sub>2</sub> (a stable analogue of thromboxane A<sub>2</sub> mimetic) [7] was more sensitive in endothelium-intact or denuded strips, suggesting that the up-regulation of thromboxane A<sub>2</sub> may be independent on the disorder of endothelial function in preeclampsia [8]. Thus it needs to be clarified with the increase responses to various vasopressors, including angiotensin II and thromboxane A<sub>2</sub> in preeclampsia.

## ENDOTHELIUM-DERIVED RELAXING FACTORS (EDRF)

Recently, it has been suggested that under placental site hypoperfusion and maternal vascular complication, the

\*Address correspondence to this author at the Department of Obstetrics and Gynecology, Graduate School of Medical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467-8601, Japan; Tel: +81-52-8538241; Fax: +81-52-8422269; E-mail: og.yo@med.nagoya-cu.ac.jp

reduced uteroplacental perfusion might develop endothelial cell activation or damage in patients with preeclampsia [9].

Vascular endothelial cells release EDRF, such as nitric oxide (NO) [10], prostacyclin [11] and endothelium-derived hyperpolarizing factor (EDHF) [12, 13] and these play important roles in the regulation of vascular tone, vascular permeability and blood coagulation, thus helping to maintain circulatory homeostasis [14].

The reduced actions of EDRF may develop endothelial cell activation and establish preeclampsia. It is ascertained that in management of preeclampsia, it is important to normalize the endothelial cell activation-damage. There is now increasing evidence to implicate the vascular endothelium both in the cardiovascular adaptation to normal pregnancy and in the pathogenesis of preeclampsia.

#### **ENDOTHELIUM-DERIVED NITRIC OXIDE (NO)**

When agonist or shear stress increases the concentration of intracellular calcium, upon calcium-induced binding calmodulin activates endothelial NO synthase (eNOS) in the endothelium. The activated eNOS catalyzes electron transfer from nicotinamide adenine dinucleotide phosphate dehydrogenase (NADPH) to L-arginine and generates NO and L-citrulline in the presence of the co-factor tetrahydrobiopterin under physiological conditions. NO produced from the endothelium increases the cellular concentration of guanosine-3',5'-cyclic monophosphate (cGMP) through activation of soluble guanylate cyclase, which in turn leads to activation of protein kinase G (PKG) and thus produces a smooth muscle relaxation [14].

Non-invasive techniques, for evaluating flow-mediated vasodilatation (FMD) induced by reactive hyperemia in the brachial artery [15] have shown production of NO to be increased in endothelium by shear stress [16-18]. During pregnancy, increase in production of NO may be induced by estrogens [19,20]. Recently, 17 $\beta$ -estradiol is found to enhance vasorelaxation due to increase in production of NO through the Akt/PKB pathway [21]. The FMD in normotensive pregnant women is reported to be larger than in non-pregnant women [22, 23]. However, *in vivo* investigations have shown that FMD is reduced in patients with preeclampsia [23, 24].

With use of omental resistance arteries, the action of endothelium-induced NO is less pronounced in endothelium-intact strips obtained from preeclamptic women than those from normotensive pregnant women. Furthermore, the relaxation of STA<sub>2</sub>-induced contraction by both sodium nitroprusside (SNP, a NO donor) and 8pCPT-cGMP (a membrane permeable, phosphodiesterase-resistant cGMP analogue) is attenuated in the endothelium-denuded strips from preeclamptic women in comparison with those from normotensive pregnant women. Taken together, these available results indicated that reduced action of endothelial NO might be due to a decrease in the action of NO itself, especially decrease in activity downstream of cGMP [8].

Recently, evidence has accumulated that the production of NO in the endothelial cells may in fact be increased in preeclampsia [25-29]. It has also been found that in arteries from subcutaneous fat, endothelial NO synthase expression

is increased in patients with preeclampsia [30]. Furthermore, the concentration of cGMP in platelet obtained from patients with preeclampsia is not different from that in normotensive pregnant women, although FMD by hyperemia is reduced in preeclampsia [23]. Many evidences thus supported that the production of NO in the endothelium is not reduced in preeclampsia.

Interestingly, it was found that in peripheral resistance artery angiotensin II inhibits the action of cGMP via angiotensin II type 1 receptor (AT<sub>1</sub>R) [31]. In conduit arteries obtained from nitroglycerin (NTG) treated rabbit, AT<sub>1</sub>R developed the cross tolerance of NTG and reduced the action of endothelial NO due to production of superoxide [32-34].

It is supposed that the increased production of superoxide directly or *via* activated AT<sub>1</sub>R reduced the action of cGMP or protein kinase G (PKG; a target molecule of cGMP) in addition to increased response to angiotensin II in resistance artery in preeclampsia like a NTG cross tolerance, although the production of NO is not reduced in the endothelium (Fig. 1).

#### **ENDOTHELIUM-DERIVED PROSTACYCLIN**

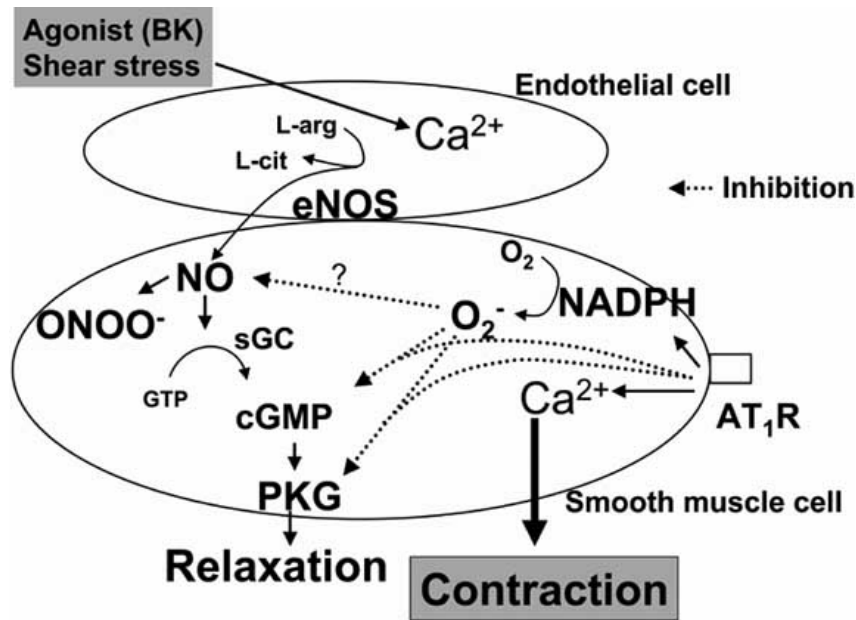
Prostacyclin is synthesized in vascular endothelium and is known to be vasodilator as well as powerful inhibitor of platelet aggregation [11]. During pregnancy, the synthesis of 6-keto-PGF<sub>1</sub> (a stable prostacyclin metabolite) has been found to be increased in fetoplacental and umbilical tissues and is thought to be important role in the regulation of not only the maternal but also the fetal blood circulation [35]. In preeclampsia, the urinary and blood concentrations of 6-keto-PGF<sub>1</sub> are significantly decreased [36, 37].

Although bradykinin increased the concentration of 6-keto-PGF<sub>1</sub> in both preeclamptic and normotensive groups of women, the concentration of 6-keto-PGF<sub>1</sub> in the absence and presence of bradykinin was significantly lower for preeclamptic women. In contrast, on treatment with beraprost (a stable prostacyclin analogue), both relaxation of STA<sub>2</sub>-contraction and hyperpolarization in the smooth muscle cells in omental resistance arteries were found to be identical between two groups of women [38]. These results could directly confirm previous findings [36, 37].

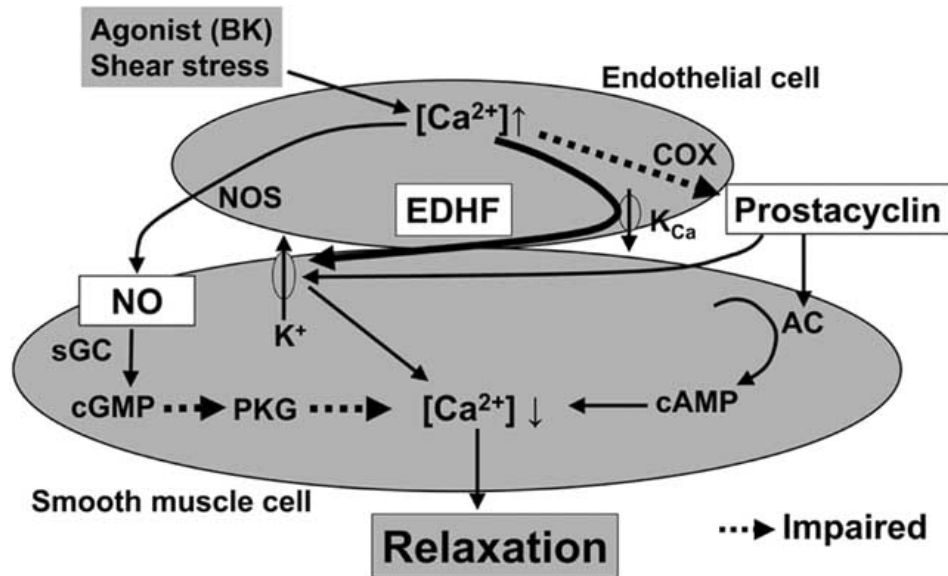
#### **ENDOTHELIUM-DERIVED HYPERPOLARIZING FACTOR**

The cyclooxygenase inhibitor and NO synthase inhibitor independent dilator responses, named as endothelium-derived hyperpolarizing factor (EDHF), are associated with vascular smooth muscle hyperpolarization. The identification of EDHF is unsolved. Possible candidate factors were epoxides of arachadonic acids (epoxyeicosatrienoic acids; EETS) [39, 40], anandamides [41], potassium ions [42] and hydrogen peroxide [43]. There may be preferential transfer of EDHF *via* cellular gap junction [44, 45].

Accumulating evidence supports the hypothesis that EDHF may play an important role in agonist-stimulated, endothelium-dependent relaxation in pregnant resistance arteries [5, 8, 46]. It was shown that in human omental resistance arteries, bradykinin produces an endothelium-dependent relaxation through actions of NO- and



**Fig. (1).** Schematic illustration of possible mechanisms underlying characteristic changes in NO in preeclampsia. The increased production of superoxide directory or via activated AT<sub>1</sub>R reduced the action of cGMP or PKG in addition to increased response to angiotensin II in resistance artery in preeclampsia. BK; bradykinin, NOS; NO synthase; L-arg; L-arginine, L-cit; L-citrulline, NADPH; nicotinamide adenine dinucleotide phosphate dehydrogenase, sGC; soluble guanylyl cyclase, PKG; protein kinase G, ONOO<sup>-</sup>; peroxyntate, AT<sub>1</sub>R; angiotensin II type1 receptor.



**Fig. (2).** Schematic illustration of possible mechanisms underlying characteristic changes in EDRF in preeclampsia. 1) the action of endothelial NO is reduced not due to decrease in the production of NO in the endothelium but rather to reduced action of cGMP itself and/or downstream of cGMP, 2) production of prostacyclin is reduced in endothelium and 3) the action of EDHF is preserved. BK; bradykinin, NOS; NO synthase; COX; cyclooxygenase, sGC; soluble guanylyl cyclase, AC; adenylyl cyclase, PKG; protein kinase G.

prostacyclin-independent factors [5]. Since this could be blocked by charybdotoxin (CTX; a blocker of intermediate- and large-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels) plus apamin (small-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels), it

was suggested that endothelium-dependent relaxation is mediated by actions of EDHF [8], although no direct proof for bradykinin-induced membrane hyperpolarization in human pregnant resistance arteries is lacking.

Recently, it was found that in omental resistance arteries from pregnant women, bradykinin does indeed produce a smooth muscle cell membrane hyperpolarization, which is sensitive to CTX plus apamin. The magnitude of the hyperpolarization mediated by EDHF in omental resistance arteries in preeclampsia, however, is similar to the response seen in normotensive pregnant women. Thus, as previously agreed, the function of EDHF may remain normal in resistance arteries in preeclampsia [38].

## SUMMARY

On *in vitro* investigation of characteristic changes in preeclampsia using vascular strips of omental resistance artery obtained from preeclamptic women, it was found that: 1) the action of endothelial NO is reduced not due to decrease in the production of NO in the endothelium but rather to reduced action of cGMP, a second messenger of NO, itself and/or downstream of cGMP, 2) production of prostacyclin in endothelium is reduced, and 3) the action of EDHF is preserved (Fig. 2).

The characteristic changes seen in endothelial function in preeclampsia appear to be very different from the endothelial damage that occurs with vascular diseases, such as hypertension and diabetes, since EDHF might work to compensate for both the reduced action of endothelial NO and the reduced production of prostacyclin in resistance arteries in preeclampsia.

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