Tohoku J. Exp. Med., 2018, **244**, 243-248

Invited Review

Vitamin B_3 Nicotinamide: A Promising Candidate for Treating Preeclampsia and Improving Fetal Growth

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Up to 8% of pregnant women suffer from preeclampsia (PE), a deadly disease characterized by high blood pressure (BP), blood vessel damage, called endotheliosis (vascular endothelial swelling with narrowing of capillary lumen), and high levels of protein in the urine. PE is often associated with premature delivery, which is a risk factor of cardiovascular and metabolic diseases among the offspring. Accordingly, establishing drug treatments of PE is in immediate needs. Currently, many of anti-hypertensive drugs cause malformation of the fetuses and are contraindicated for pregnant women. Anti-hypertensive drugs that are allowed to be used for treating pregnant women could lower BP of the mothers and reduce the risk of maternal death due to cardiovascular diseases such as cerebral hemorrhage. However, these anti-hypertensives do not improve endotheliosis and proteinuria. In fact, they reduce blood supply to the placentae and fetuses, which could lead to fetal growth restriction (FGR) and fetal and neonatal death. Until now, the only treatment for preeclamptic women has been delivery of the baby and placenta. Using three mechanistically different mouse models of PE, we have found that vitamin B_3 nicotinamide (Nam) is the first safe drug that alleviates PE, and that Nam also alleviates or prevents miscarriage, prolongs pregnancy period, and improves the growth of the fetuses in mice with PE. Importantly, Nam has been used for pregnant and nursing women who have difficulty in taking sufficient meal. Nam could help treat or prevent PE and FGR associated with PE, if the treatment works in humans.

Keywords: endotheliosis; fetal growth restriction; nicotinamide; preeclampsia; premature delivery
Tohoku J. Exp. Med., 2018 March, **244** (3), 243-248.

Introduction

Preeclampsia (PE) is a disease in pregnant women, characterized by high blood pressure (BP) and proteinuria (Levine et al. 2004). PE often rapidly deteriorates and causes maternal death due largely to cardiovascular diseases. It sometimes also leads to fetal growth restriction (FGR) and fetal or neonatal death. The number of PE patients is increasing with increasing age of child bearing women. There are about 20,000 pregnant women who develop PE in a year in Japan. It is estimated that about 4,000,000 women annually develop PE in the world. However, the fundamental treatment of PE is not established. PE is a risk factor of future cardiovascular diseases. PE is often associated with premature delivery, which is a high risk in the offspring of neurodevelopmental impairment (Sutton and Darmstadt 2013) and of cardiovascular and metabolic diseases (Nuyt et al. 2017). Accordingly, establishing drug treatments of PE is in immediate needs.

Received January 19, 2018; revised and accepted March 8, 2018. Published online March 20, 2018; doi: 10.1620/tjem.244.243.
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Dr. Nobuyuki Takahashi is a recipient of the 2016 Gold Prize, Tohoku University School of Medicine.

The Problem in Treating PE

Other than high BP and proteinuria, endotheliosis, swelling of endothelial cells and narrowing of capillary lumen, is also a characteristic feature of PE (Fig. 1). The problem in treating PE is that there is no effective drug treatment to save both mothers and babies. Different kinds of anti-hypertensives are clinically used. However, many of them cause malformation of the fetuses and cannot be used for pregnant women. Anti-hypertensives we can use for pregnant women can save PE mothers from cardiovascular diseases. However, because they do not improve endotheliosis, lowering maternal BP reduces placental and fetal blood flow, and leads to poor prognosis of the baby (Kingman et al. 2009; von Dadelszen et al. 2000). Until now the only way to solve this problem is to induce delivery of the premature baby and placenta, which secretes anti-angiogenic factors. Because delivery of premature baby could affect neuropsychological development, and is the risk of developing cardiovascular diseases and the metabolic syndrome in the future, it is extremely important to establish drug treatment of PE that not only reduces maternal BP, but also prolongs pregnancy period, and improves fetal growth that secures fetal/neonatal health.

Pathogenesis of PE

To develop novel treatments, it is necessary to understand the pathogenesis of PE. The most widely accepted understanding of the pathogenic mechanism of PE is that PE is primarily the consequences of an imbalance between pro-angiogenic growth factors that keep vascular health (such as vascular endothelial growth factor, VEGF), and anti-angiogenic factors, such as the soluble form of VEGF receptor-1 (sVEGFR-1, commonly referred to as sFlt-1) (Fig. 2) (Levine et al. 2004). sFlt-1 is a splice variant of VEGFR-1, which lacks transmembrane domain and cytosolic domain, and is secreted from trophoblasts of the placenta into the circulation. sFlt-1 has ligand-binding domain, traps VEGF, and works as its antagonist. Both the hypertension and the proteinuria of PE are caused by abnormally high amounts of anti-angiogenic factors derived from the placenta. FGR is often associated with PE, and is a consequence of reduced placental blood flow by anti-angiogenic factors and/or to impaired development of the placenta.

The decrease in the levels of VEGF activates the endothelin system. We have previously shown that the renal levels of endothelin-1 (ET-1), the most powerful naturally occurring pro-hypertensive peptide, and of the endothelin type A receptor (ET_AR) are increased by excessive sFlt-1 (Murphy et al. 2010; Li et al. 2012). Antagonists of ET_AR greatly ameliorate the PE-like condition that develops in the kidneys with experimentally induced excessive sFlt-1 (Murphy et al. 2010; Li et al. 2012). Unfortunately, mice lacking ET-1 and ET_AR are lethal, and ET_AR antagonists are teratogenic and contraindicated for use in pregnant women (Kingman et al. 2009). Consequently, we cannot use this class of drugs to treat PE.

Expected effect of Vitamin B_3 Nicotinamide on PE

Although inhibiting ET_AR is not suitable for pregnant women, inhibiting some of ET-1 signaling could help treat PE without adverse effects. Nam does not cause malformation of the fetuses, and inhibits vasoconstriction by ET-1 through inhibiting adenosine diphosphate (ADP) ribosyl cyclase, and reducing its product cyclic ADP ribose and Ca^{2+} mobilization (Fig. 3) (Thai and Arendshorst 2008; Arendshorst and Thai 2009).

Moreover, nicotinic acid increases heme oxygenase-1 (HO-1) expression, which sequentially increases VEGF (Dulak et al. 2004), increases endothelial nitric oxide synthase (eNOS) expression and activity, and decreases BP (Fig. 4). Vitamin B_3 nicotinamide (Nam) likely has the similar effects on HO-1 expression and BP. HO degrades

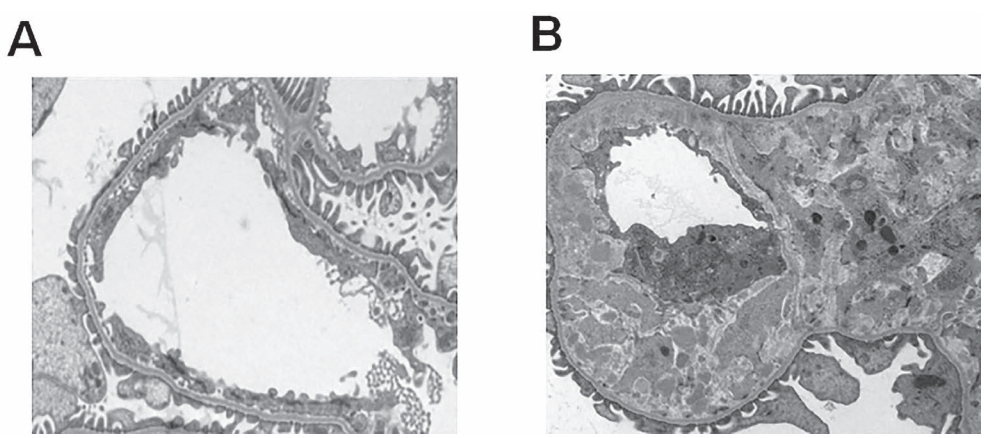

Fig. 1. Endotheliosis of kidney glomerular capillary.
Glomerular capillary from a control mouse (A). Glomerular capillary from a preeclamptic mouse overexpressing the soluble form of VEGF receptor-1 (sVEGFR-1, commonly referred to as sFlt-1) sFlt-1 (B). Glomerular capillary in the kidney from preeclamptic mice has characteristic feature of endotheliosis: swelling of endothelial cells and narrowing of capillary lumen.

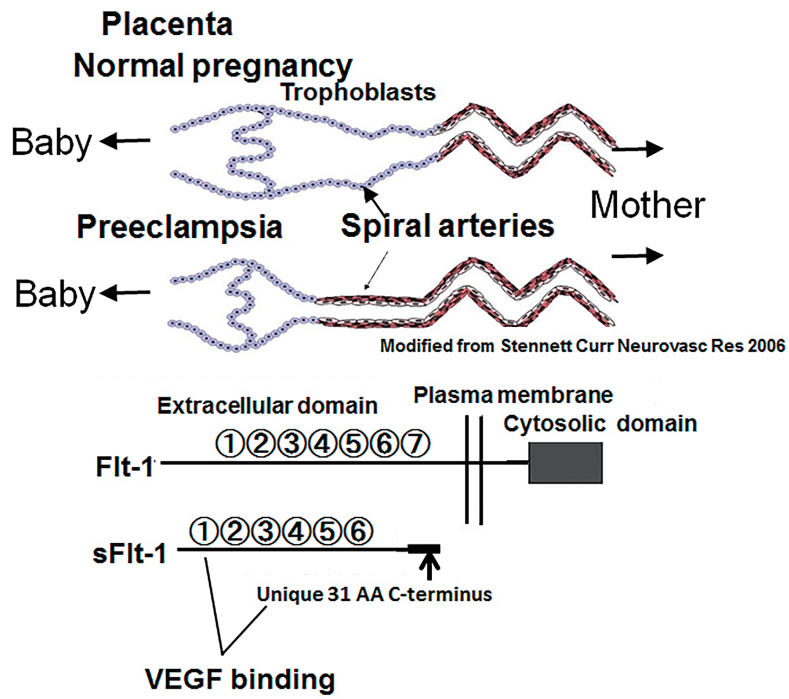

Fig. 2. Pathogenesis of preeclampsia.
The pathogenesis of preeclampsia (PE) is not fully known, but when the invasion of trophoblast into spiral arteries is poor, flaccid low resistance arteries do not develop, and placenta and fetus become ischemic, which increases the expression of sFlt-1 in the placenta. sFlt-1 is the soluble VEGF (vascular endothelial growth factor) receptor 1, which lacks transmembrane and cytosolic domains, and is secreted into the circulation. Because sFlt-1 has ligand binding domain, VEGF binds to sFlt-1, and cannot bind to full length VEGF receptor 1 (VEGFR1) and VEGFR2. sFlt-1 works as an antagonist of VEGF. sFlt-1 overexpression model using adenovirus is a wildly used model of PE.

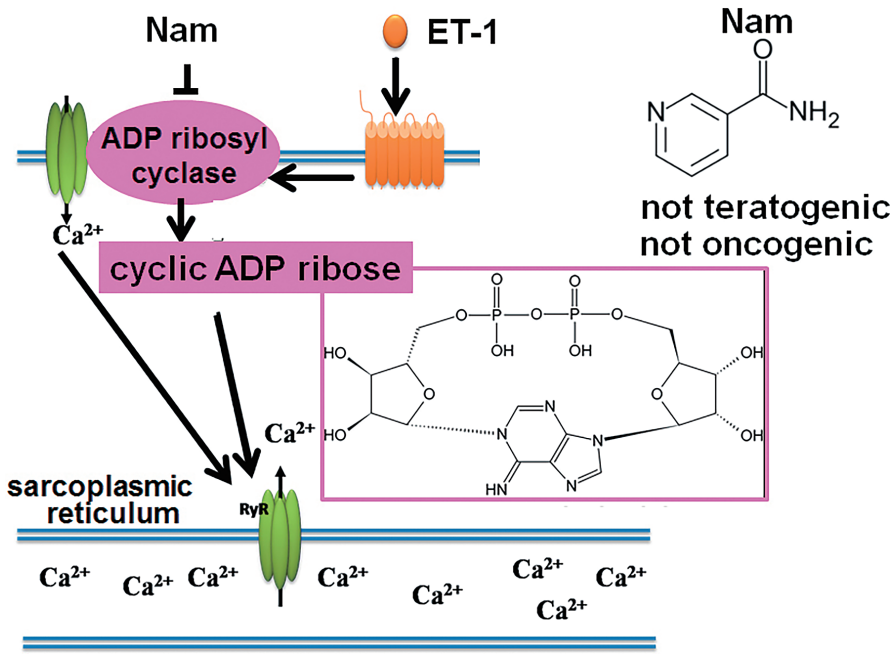

Fig. 3. Nicotinamide (Nam) inhibits vasoconstriction by endothelin (ET-1).
Although inhibiting type 1 ET receptor (ETAR) is not suitable for pregnant women, inhibiting part of its signaling could help treating PE without adverse effects. Nam is known to inhibit vasoconstriction by ET-1 through inhibiting adenosine diphosphate (ADP) ribosyl cyclase, and reducing its product cyclic ADP ribose (cADPR) and Ca^{2+} mobilization. Because Nam is not teratogenic, it would be a promising drug to treat PE patients. Modified from Thai and Arendshorst (2008).

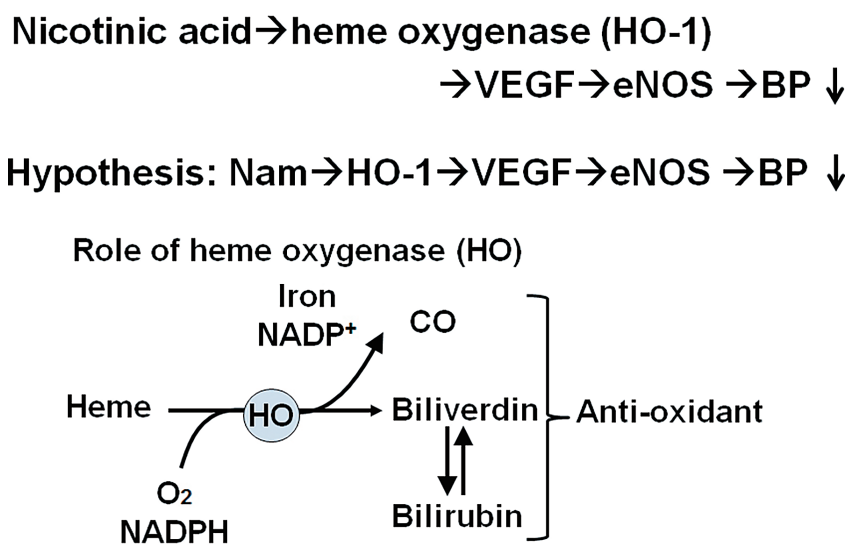

Fig. 4. Nam is anti-oxidant and likely decreases BP.
Nicotinic acid increases heme oxygenase-1 (HO-1) expression, and decreases BP. The same could be true for Nam. HO degrades heme and produces carbon monoxide and biliverdin/bilirubin. These are antioxidants, and Nam likely ameliorates PE by reducing oxidative stress.

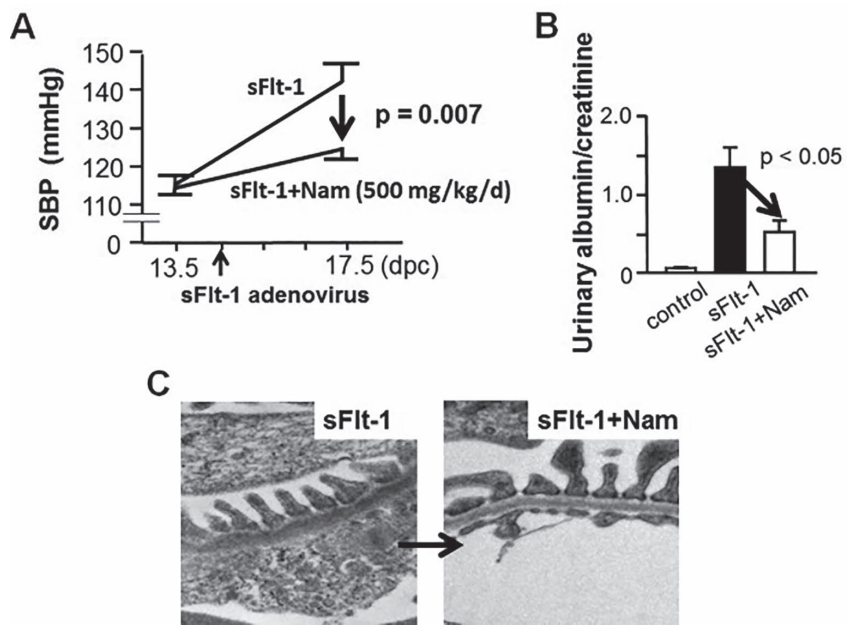

Fig. 5. Nam ameliorates PE induced by sFlt-1 in mice.
sFlt-1 increases BP and urinary albumin excretion. Nam inhibited these changes, and corrected endotheliosis. Modified from Li et al. (2016).

heme and produces carbon monoxide and biliverdin/bilirubin. Nicotinamide adenine dinucleotide phosphate (NADPH) is required for this reaction. Nam becomes nicotinamide adenine dinucleotide (NAD^+), and NAD^+ kinase in cytosol and mitochondrion changes NAD^+ to $NADP^+$. $NADP^+$ becomes NADPH by the transfer of reducing equivalent from NADH to $NADP^+$. Because oxidative stress and inflammation causes FGR, Nam is a promising drug to ameliorate PE, premature delivery, and FGR by its anti-oxidative property.

Effects of Nam on PE in Mouse Models

To test our hypothesis that Nam ameliorates PE, premature delivery, and FGR, we evaluated the effects of Nam on PE using three mechanistically different mouse models. We first used excessive sFlt-1 model by overexpressing sFlt-1 using adenovirus. In this model Nam alleviated all of the characteristic features of PE: hypertension, proteinuria, and endotheliosis (Fig. 5) (Li et al. 2016). Moreover, Nam prolonged the pregnancy period, reduced miscarriage

rate and premature delivery, and corrected FGR (Fig. 6). Nam corrects hypertension at least partly by inhibiting ADP ribosyl cyclase. Nam prolongs pregnancy period, alleviates FGR likely through improving mitochondrial function and ATP production (Li et al. 2016).

The second mouse model of PE is the mice lacking *Asb4* (Ankiryn-repeat-and-suppressor of cytokine signaling (SOCS)-box-containing-protein 4). These mice have a problem in implantation of embryos and development of placenta, and causes PE and fetal death (Townley-Tilson et al. 2014). In these mice Nam improves the characteristic features of PE, prolongs the pregnancy, and increased fetal survival rate (Li et al. 2016).

The third mouse model of PE is reduced uterine perfusion pressure (RUPP) model. RUPP model in rats has been widely used, and is produced by surgically ligating arteries to the uterus. To investigate the role of genes on PE, we need to study using mice, because there are already many different kinds of genetically engineered mice. However, it has been difficult to develop mouse RUPP model. We developed a mouse RUPP model for the first time by ligating vessels feeding uterus, placenta and fetuses with nylon thread, and immediately removing it to give a space in the vessels for blood to flow (Fig. 7) (Fushima et al. 2016). Using this mouse RUPP model, we have confirmed that Nam also alleviates PE, premature birth, and FGR (Fushima et al. 2017).

Perspectives

Nam is water-soluble vitamin B_3 and used for treating

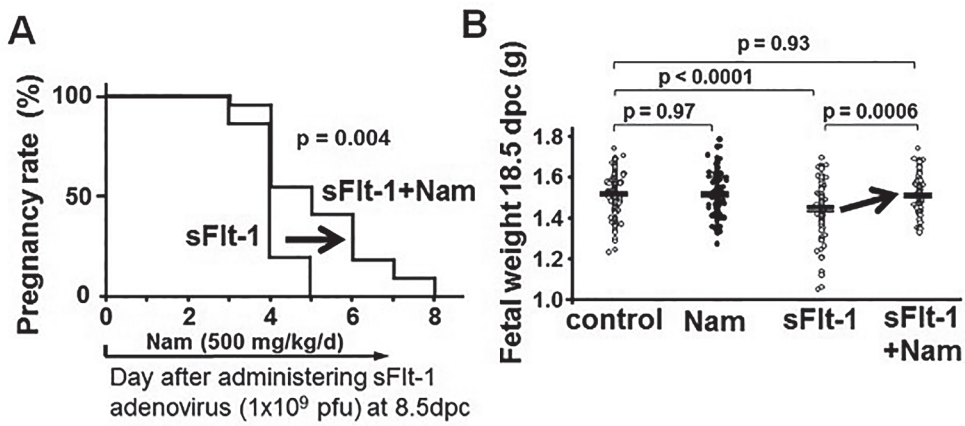

Fig. 6. Nam prolongs pregnancy duration and corrects fetal growth restriction (FGR).
Nam prolongs pregnancy period. Nam does not change body weight of fetuses in the absence of sFlt-1. But when sFlt-1 is overexpressed fetal body weight decreased and Nam corrected it to the control levels. Modified from Li et al. (2016).

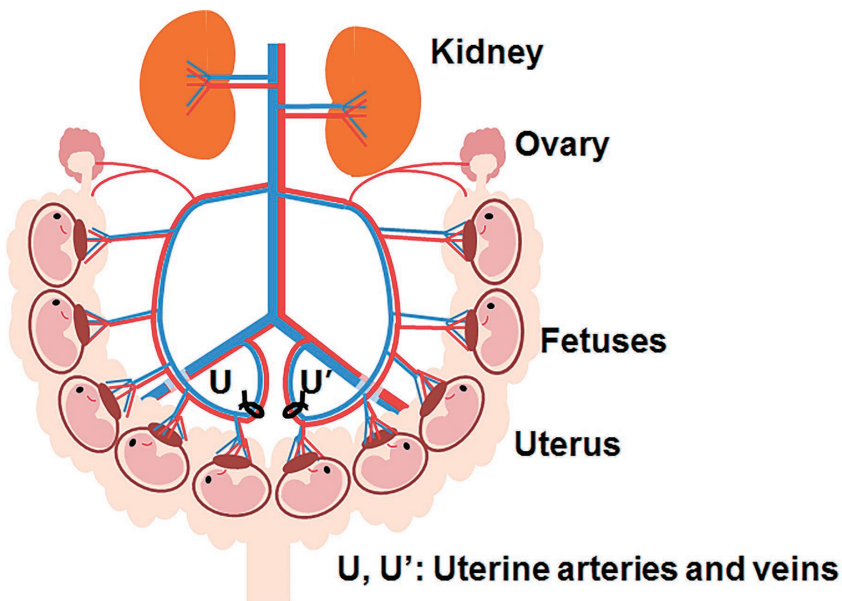

Fig. 7. Mouse reduced uterine perfusion pressure (RUPP) model of PE.
Uterine arteries and veins (U, U') are constricted to reduce uterine blood flow. Modified from Fushima et al. (2016).

various diseases, such as pellagra, bullous pemphigoid, schizophrenia, depression, insomnia, and Alzheimer's disease. It is a safe drug and is also used for pregnant and nursing women who have difficulty in taking sufficient meal (Knip et al. 2000). In the three mouse models of PE that recapitulate human PE, we have demonstrated that Nam is the first drug that improves and prevents all of hypertension, proteinuria, endotheliosis, miscarriage/premature birth, and FGR. After confirming the safety and efficacy of Nam to preeclamptic women and successful clinical trials, indication of Nam should be expanded to treatment and prevention of PE. Nam is likely to save both mothers and fetuses, to improve development, and to prevent the metabolic syndrome and cardiovascular diseases of the babies all over the world because it is inexpensive.

Acknowledgments

We thank H.W. Davin Townley-Tilson, Hironobu Nakada, John R. Hagaman, Jennifer Wilder, and Manyu Li, J. Charles Jennette, Nobuyo Maeda, and Oliver Smithies.

Our work was supported by a Grant-In-Aid from the Japan Society of Promotion of Science (JSPS, 24659409), Translational Research Network Program of Ministry of Education, Culture, Sports, Science and Technology of Japan (J140001192), a major grant from the National Institutes of Health (HL049277), funds from the Department of Pathology and Laboratory Medicine at UNC Chapel Hill, the Naito Foundation and Miyagi Kidney Foundation.

Conflict of Interest

The authors declare no conflict of interest.

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