EXPERT OPINION

New insights into the role of aminopeptidases in the treatment for both preeclampsia and preterm labor

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Introduction: Evidence elucidating the pathophysiology and pharmacology of conventional drugs, β-2 stimulants and magnesium sulfate, on safety and effectiveness for preeclampsia and preterm labor are rarely found. Both compounds pass through the placental barrier and could exert their adverse effects on the fetus. Exposure to these agents could be problematic long after the birth, and possibly result in diseases such as autism and cardiomyopathy. Since 1970 the possible roles of placental aminopeptidases, which degrade peptide hormones, in preeclampsia and preterm labor have been studied.

Areas covered: Many studies reveal that the fetus secretes peptide hormones, such as angiotensin II, vasopressin, and oxytocin, under hypoxia (stress) during the course of its growth, suggesting the critical effects these hormones have during pregnancy. The roles of placental aminopeptidases, the enzymes which degrade fetal hormones without passing through the placental barrier, were clarified. A first-step production system for recombinant aminopeptidases was established, by which engineered recombinant aminopeptidases were used for further experiments testing expected efficacy on controlling the level of hormones.

Expert opinion: The authors conclude that both aminopeptidase A and placental leucine aminopeptidase could be potentially safe and effective drugs for patients and their babies in the treatment of preeclampsia and preterm labor.

Keywords: aminopeptidase A, angiotensin, autism, β-2 stimulant, cardiomyopathy, fetal peptide hormone, magnesium sulfate, oxytocin, placental leucine aminopeptidase, preeclampsia, preterm labor, vasopressin

Expert Opin. Investig. Drugs [Early Online]

1. Introduction

Management of obstetric disorders such as preeclampsia and preterm labor requires not only efficacy and safety for a pregnant patient, but also caution on the safety of the fetus.

Commonly used antihypertensive drugs, angiotensin-converting enzyme inhibitor (ACE) and angiotensin subtype 1 receptor blocker (ARB), are contraindicated for pregnant women because these drugs are toxic to the fetus [1].

There are limited drugs currently used for preeclampsia and preterm labor; furthermore, these drugs are often neither effective enough nor safe for the fetus. Immediate cure for the patients of preeclampsia or eclampsia often calls for the artificial delivery of premature babies irrespective of the gestational age [2].

Magnesium sulfate ($MgSO_4$) is widely used for preeclampsia and preterm labor; however, this compound is essentially an anticonvulsant, having little effect on both normalizing blood pressure (BP) and inhibiting uterine contraction. In addition,

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Article highlights.

- The onset of labor is induced by excess efflux of fetal OT, which accompanies fetal growth, into the maternal circulation without being degraded by P-LAP.
- Degradation of A-II by APA before binding to the receptor 1 subtype to achieve BP lowering is a plausible approach.
- Novel genetically engineered aminopeptidases (APA) were successfully produced on a mass scale using the bacurovirus system, with which the effective dose is 2300-fold greater than candesartan by molecule-based comparison.
- Currently used drugs to treat preeclampsia such as β-2 stimulants and $MgSO_4$ can pass the placental barrier and exerts toxic effects on the fetus, which may persist after birth, with a suspected connection to infant dilated cardiomyopathy.
- In light of a lack of safe and effective medication, recombinant APA and recombinant P-LAP (using Chinese hamster ovary cells) should be prioritized for pharmaceutical development.

This box summarizes key points contained in the article.

one study has revealed that the fetal exposure to magnesium sulfate was associated with poor development of the blood vessels in the pup's cardiac tissue in the pregnant rat model [3].

The objectives of this review are, first, to explain the underlying mechanisms on BP regulations in preeclampsia as compared with normal pregnancies, in particular to focus on fetal angiotensin II (A-II) and a specific A-II-degrading enzyme called aminopeptidase A (APA, EC3.4.11.7). The regulating mechanisms of uterine contraction in preterm labor and spontaneous onset of labor in normal term are also explained. Fetal oxytocin (OT), arginine vasopressin (AVP), and the enzyme capable to degrade these hormones called placental leucine aminopeptidase (P-LAP) (EC3.4.11.3) will be highlighted. APA and P-LAP have been studied on a clinical and laboratory basis since 1970 [4].

Secondly, the adverse effects of conventionally used drugs for preeclampsia and preterm labor on fetus and neonates will be referred to and anticipated long-term prognosis of newborns exposed to these drugs will be discussed.

Finally, innovative treatment strategies with recombinant APA (rec APA) for preeclampsia and P-LAP (rec P-LAP) for preterm labor will be introduced. Both are expected to be significantly safer and more effective medications for pregnant patients and their babies, compared with high-risk conventional remedies.

2. Placental aminopeptidases are indispensable for degradation of fetal peptide hormones

It is well known that the fetal peptide hormones such as A-II [5,6], AVP [7,8], and OT [7] are highly vasoactive and uterotonic. Chard *et al.* [9] have shown marked elevation of OT during labor, extracted from the umbilical artery, suggesting the involvement of feto-placental OT in inducing the onset of delivery. Oosterbaan and Swaab [8] reported that OT concentrations in the umbilical artery were higher than those in the umbilical vein and those in the maternal peripheral plasma, in both elective cesarean section and normal vaginal delivery (before and during delivery). Devane and Porter reported that increased amniotic AVP levels indicated fetal acidosis [7]. Broughton, Pipkin, and Symonds [5] reported a significant difference in A-II concentrations comparing umbilical venous and arterial blood during preeclampsia, suggesting increased A-II release from the stressed or transiently hypoxemic fetus. Kingdam *et al.* [6] observed a relationship between umbilical arterial oxygen partial pressure and A-II levels in the umbilical vein during delivery, suggesting increases in A-II levels under hypoxic environments.

These results indicate that under stress A-II and AVP levels are elevated in the human fetus. As these hormones readily cross the placental barrier they are capable of exerting their effects on the mother's physiology. The feto-placental unit is a rapidly growing organ; therefore, increases in maternal exposure to fetal hormones via retro-placental maternal blood supply are intensified with advancing pregnancy. Thus, fetal peptide hormones are potentially active not only within the placenta, but also in the maternal circulation. The significant influence of aminopeptidases on these feto-placental peptides in the maternal barrier has been identified: P-LAP (EC3.4.11.3) that acts on OT [10,11]; and AVP and APA (EC3.4.11.7) that act on A-II (**Figure 1**) [12,13].

3. Angiotensinase

APA is a naturally produced protein (enzyme) from human organs including the placenta. Its property of degrading A-II is conferred in the blood circulation converting it into angiotensin III (A-III) with an approximately 20% potency compared with A-II, and thus it normalizes BP. Recently, the classical view of the renin-angiotensin system (RAS) has been challenged by the discovery of the enzyme ACE2 [14,15], in addition to the increasing awareness of many angiotensin peptides other than A-II having biological activities and physiological importance [16,17].

ACE2 cleaves angiotensin I (A-I) to angiotensin-(1-9) (Ang 1-9) and cleaves A-II to angiotensin-(1-7) (Ang 1-7) [18]. The reported vasodilative actions of Ang 1-7, along with the potential involvement of ACE2 in both A-II degradation and Ang 1-7 production, add complexity to the RAS. However, in contrast to these *in vitro* studies, Campbell *et al.* reported the role of ACE2 in A-I metabolism and showed negative evidence regarding Ang 1-9 as an important precursor for Ang 1-7 formation in human studies. Heart failure subjects receiving ACE inhibitor had > 40-fold increase in A-I levels; however, Ang 1-9 levels were low (1–2 fmol/ml) and similar to those of normal subjects. Ang 1-7 levels

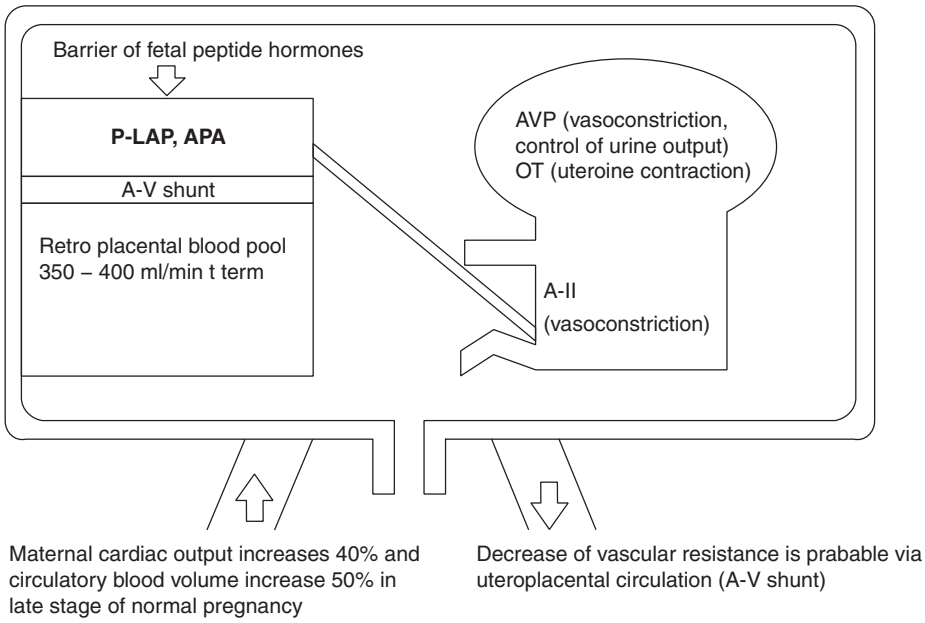

Figure 1. Placental aminopeptidases are involved in homeostasis of BP and uterine-tonus via regulating fetal peptide hormones.

increased in parallel with A-I levels and Ang 1-7/A-II ratio increased by 7.5-fold in coronary sinus blood. Administration of ACE inhibitor to these patients rapidly decreased A-II levels by approximately 60% and increased A-I levels by approximately 2.6-fold. However, the compound did not alter Ang 1-7 levels. The failure of Ang 1-9 levels to increase in response to increased A-I levels indicates negative evidence for ACE2 in A-I metabolism. The levels of Ang 1-7 were more linked to those of A-I than A-II, suggesting that the pathway from A-II to Ang 1-7 may be negligible. Their data suggest against a role for Ang 1-9 as an important precursor for Ang 1-7 formation. Their data should be evaluated for the active role of the ACE2 pathway in RAS in humans, although they mentioned that their data are limited to the coronary circulation (**Figures 2** and **3**). We should wait for a definite conclusion on whether contribution of ACE2 in RAS is truly crucial in humans or not [19].

It has long been perceived that APA acts as a rate-limiting factor under existence of the potent vasoconstrictor A-II [13,20]. A study of APA knockout (KO) mice revealed that increased BP was possibly due to elevated A-II concentrations in the animals [21].

Pregnancy in humans and animals is normally characterized by a relative refractoriness to the hypertensive response associated with exogenously infused A-II. A-II sensitivity in the preeclampsia patient becomes higher, reaching a level equivalent to, or greater than, that of nonpregnant women [22]. These phenomena have been extensively studied, and most likely attribute to the cause of preeclampsia, although this remains controversial.

Studies using APA KO mice solved this question. APA KO mice are hypersensitive to exogenously administered A-II, strongly suggesting the involvement of APA in this A-II sensitivity [21]. It was suggested that in APA KO mice tissue, A-II concentrations were increased and thus resulted in BP elevation. Although other enzymes, including neutral endopeptidase (EC3.4.24.11) [23], prolylendopeptidase (EC3.4.21.26) [24], and ACE2 [14,15], are able to metabolize A-II *in vitro*, they do not compensate for the loss of APA [21]. APA appears to be an essential enzyme within the RAS for the regulation and maintenance of BP.

4. Oxytocinase

OT is degraded in the serum and placenta of pregnant women; hence, it is important to regulate OT activity. OT has a cysteine ring at its N-terminus, which is an essential site for exerting its uterotonic activity [25]. Three proteases have been reported to degrade OT and AVP: P-LAP, prolylendopeptidase [24], and neutral endopeptidase [23]. P-LAP degrades a ring skeleton of peptide bond between $Cys>^{1}$ and Tyr^{2} and hydrolyzes the remaining structure sequentially starting from the amino terminus. Prolylendopeptidase and neutral endopeptidase are not involved in opening the ring skeleton of OT [11,26], which means oxytocinase should be considered as the P-LAP itself.

Administration of OT is the most effective medication for inducing labor; however, a successful efficacy depends on the stage of pregnancy when it is administered. The pregnant

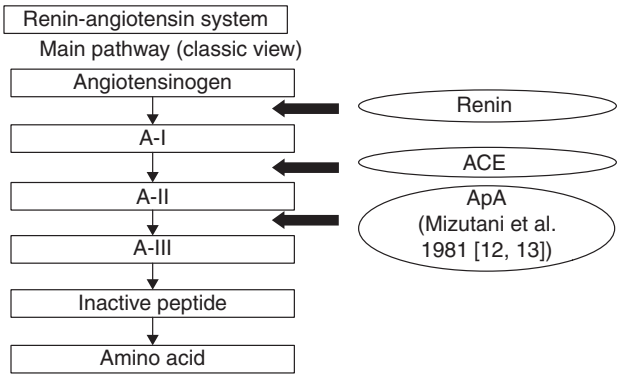

Figure 2. Components of the RAS (old pathway) Kaplan NM [69].

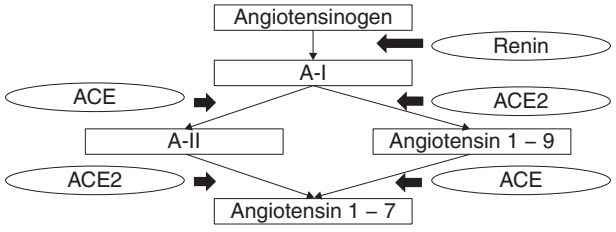

Figure 3. Updated pathway of RAS, Burrel LM *et al*. [18].

uterus becomes extremely sensitive to OT only near to the onset of labor.

Pregnant P-LAP KO mice showed increased sensitivity to exogenously administered OT, shortening of pregnancy term, and the animals delivered their pups prematurely [27]. These results suggest that P-LAP activity is involved in OT sensitivity both at term of pregnancy and the onset of labor.

Although initial characterization of OT KO mice suggested that OT was not important in the onset of labor [28,29], further studies using OT KO mice confirmed the possible interrelationship between OT and prostaglandins (PGs) in the onset of labor. Additional studies using OT KO mice have shown that the prepartum interrelationship among OT, PGs, and progesterone are important for determining precise timing of the onset of labor. Imamura *et al.* [30] reported that the labor was induced at higher progesterone levels in OT KO mice. This suggests that even under low OT receptor levels, a normal labor can occur with high serum progesterone.

The result from these studies suggests that not only OT, but also other OT-regulating substances, may be important for inducing labor. The authors also showed that OT at higher doses, without the increase in OT receptors, induced mouse preterm labor in P-LAP KO mice [27]. These results undoubtedly suggest that the regulation of the onset of labor is dependent primarily on the balance between OT and P-LAP (the OT-degrading enzyme).

5. Possibility of adverse effects on the fetus associated with conventional treatments for preeclampsia and preterm labor

Therapeutic attempts with conventional treatments show little evidence that they alter the underlying pathophysiology of preeclampsia. In fact, acute antihypertensive therapy is not used for patients of preeclampsia, as these agents can deteriorate the growth of the fetus. Acute BP control in preeclampsia may be achieved with hydralazine, labetalol, or nifedipine. Needless to say, ACE inhibitor/ARB is prohibited in pregnancy. ACE inhibitor/ARB is not only teratogenic, but ACE inhibitor/ARB also results in marked feto-placental hypotension. Hypo-perfusion in feto-placental circulation results in fetal malnutrition and hypoxia. Because conventional drugs act as anti-hypertensive agents similar to ACE inhibitors/ARB, they enter into feto-placental unit; therefore, they affect fetal circulation and result in hypotension.

Overly aggressive control of BP may compromise maternal perfusion of the intervillous space and adversely impact fetal oxygenation. Diastolic BP of ≥ 110 mmHg may cause an increased risk of cerebrovascular events in the mother; therefore, the condition is an indication for emergency delivery [31]. As mentioned earlier, conventional antihypertensive medications are not routinely administered to preeclampsia patients as these agents not only fail to reduce the risk of preeclampsia, but also exert teratogenic effects on the fetus and could cause fatal complications [32].

Although the efficacy of tocolysis has been debatable, it is generally accepted that if there was a delay of 48 h in delivery, corticosteroid may be administered for reducing fetal morbidity and mortality. Tocolytic agents include the following: magnesium sulfate, PG synthesis inhibitors, calcium antagonist, β-2 stimulants, and OT receptor antagonists [33].

Magnesium sulfate has been used extensively by obstetricians for the treatment of preeclampsia over the past two decades. Magnesium sulfate appears to be less likely to exhibit serious side effects compared with β-2 stimulants. However, dose ranges need to be strictly controlled in clinical use, as higher serum levels than therapeutic range (4 – 8 mg/dl) result in the loss of patellar reflexes and even respiratory paralysis or cardiac arrest could be induced [34].

Magnesium sulfate is essentially an anticonvulsant and exerts little change in BP. As mentioned above, the authors have found that the administration of magnesium sulfate at an equivalent dose per weight to treat preeclampsia in pregnant, spontaneously hypertensive rats (SHRs) was associated with delayed development of cardiac vessels with, possibly, an irreversible adverse effect on the fetal heart [3]. Although

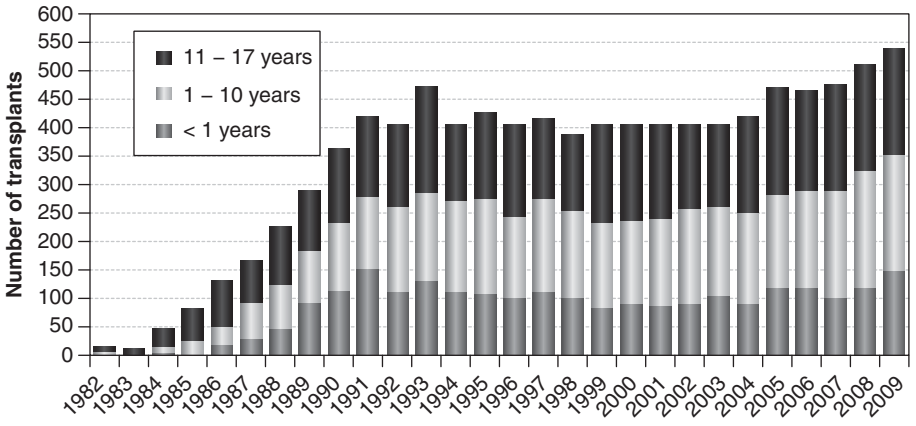

Figure 4. Age distribution of pediatric heart-transplantation recipients by year of transplant. The Registry of the International Society for Heart and Lung Transplantation: Fourteenth Pediatric Heart Transplantation Report-2011.

the majority of adverse effects of magnesium sulfate on humans are on the maternal side, it is also important to consider its impact on the fetus and neonates [35].

Both β-2 stimulants and magnesium sulfate can pass through the placental barrier and exert their effects on the fetus. In addition, treatment with β-2 stimulants presents serious maternal side effects such as arrhythmia, cardiac ischemia, and pulmonary edema, with an increased risk of tachycardia in both the mother and fetus. Furthermore, myocardial necrosis in newborns treated with terbutaline has been reported [36]. Cardiovascular function may deteriorate due to long-term administration of β-2 stimulants, which contribute to the downregulation of β-adrenal receptors of the fetus, thus resulting in impaired cardiac function following birth [37].

Cardiomyopathy is defined as the disease of cardiac muscles other than ischemic conditions. The definition excludes diseases which are secondary to congenital malformation. Although there are many studies on the genetics of dilated cardiomyopathy in children [38], about two-thirds or more of the cases are idiopathic. In other words, the etiology of infant dilated cardiomyopathy is virtually unknown.

Infant idiopathic dilated cardiomyopathy is one of the most serious heart diseases in children. The heart loses its rhythmic function due to the damage of the muscles and cannot eject the blood into the arteries. This abnormality is often identified after birth, suggesting that fetal life has been critical for developing the disease. This means any medications, which could potentially affect the fetal heart, should be considered as related to the disease.

Concern is mounting over the adverse effects of β-2 stimulant and magnesium sulfate on the fetal heart. Since the 1970s, obstetricians have used β-2 stimulants intensively. After 1990, magnesium sulfate has been used intensively for the treatment of preeclampsia and reversal of preterm labor. Heart transplantation is considered the effective cure for idiopathic dilated cardiomyopathy in infants.

Recent advancements in heart surgery with new immunosuppressive drugs together with the organ donor system have contributed to the increased number of infant heart transplantations (**Figure 4**). Epidemiological studies may be required to investigate what drugs have been used for treating the patients during pregnancy.

Witter *et al.* reported that the overstimulation of β-2 stimulant during critical periods of prenatal development induced a permanent shift in the neuro-balance of sympathetic-to-parasympathetic tone. This is a biologically plausible mechanism as β-2 stimulants can induce functional and behavioral teratogenesis, which explains the association with increased cases of autism spectrum disorders, psychiatric disorders, poor cognition, motor function, and school performance, and changes in BP in the offspring [39].

It is well known that there is a possible link between autism and suicide. The number of suicides in males (around age 25 – 35) significantly increased around 2000 (statistics of the Japanese Cabinet Office). This coincides with the years of their births when β-2 stimulants were intensively prescribed. The authors suggest that obstetricians and pediatricians should begin investigating the relationship between the exposure to β-2 stimulants *in utero* and the mental hygiene after birth, especially in males.

Doctor Barker attempted to clarify the suspected relationship between low birth weight and the risk of suicide [40]. He traced deaths from suicide at ages 20 – 74 and retrieved their weights at birth and at 1 year after birth. The results showed a retardation of body-weight gain after birth in the suicide cases. However, it was not demonstrated that low birth weight itself can be independently related to the rates of suicides. The results of his study conducted in 2001 in the UK did show relationships between mental disorders, such as depression, and low birth weight. Moreover, this risk was found to be higher in males [41].

Depression, one of the conditions that may be related to autism spectrum disorders, accounts for 70% of suicide cases

and is believed to induce suicidal behavior [40]. In fact, the extensive use of β stimulants for the treatment of preterm births in late 1980s to early 1990s is fully acknowledged. The United States Center for Disease Control and Prevention performed an in-depth analysis on juvenile depression cases. They performed postmortem examinations on the brains of juvenile victims from road accidents. This examination revealed abnormalities in brain cell counts, which were significantly higher than that of the normal youth. It was clearly shown that abnormal development had been progressing while they were *in utero* [42]. Facts from these studies clearly indicate that adverse effects caused by pharmaceutical substances, such as magnesium and β-2 stimulants, exposed to the brain of the fetus, continue to be harmful after the birth and such effects are irreversible.

As mentioned above, changes in the balance between fetal OT and P-LAP due to fetal growth triggers the onset of labor. OT receptor antagonists are effective in inducing uterine quiescence in various animals including humans at term. OT antagonists are effective for the treatment of preterm labor compared with β-agonists [43]. However, these agents have small molecule sizes and, therefore, can pass through the placenta and have various pharmacological effects on the fetus. Reversi *et al.* showed that the OT receptor antagonist "atosiban" inhibits cell growth. They suggested the possibility of OT antagonists as a cell-growth-inhibiting factor [44]. The drug effects on the fetus are therefore not clearly understood. Adverse effects associated with these agents with low molecular weight are problematic as they pass through the placenta and cause long-term adverse effects.

6. APA is a novel drug for hypertension that degrades circulating A-II

SHRs are widely used in cardiovascular research as a model of human hypertension. These animals offer opportunities to understand the mechanisms underlying the condition. BP can be normalized in SHRs with ARBs and ACE inhibitors, suggesting the involvement of the RAS in the hypertensive mechanism. Although SHRs do not exhibit elevated renin activity or increased plasma A-II concentrations compared with Wistar-Kyoto (WKY) rats, they have high sensitivity to A-II [45] similar to the condition of preeclampsia [22]. These results suggest that the SHRs have much higher vasocontractile sensitivity than WKY rats to A-II administered exogenously and to that of intrinsically produced A-II.

In the 1980s, APA was not recognized as an independent aminopeptidase. In 1981, the existence of APA in human placentas was confirmed by using both synthetic substrate (Asp-β naphthylamide) and by bioassay (rat BP measurement) [12]. The placental APA was then purified and characterization was performed [13]. APA is a homodimeric membrane-spanning zinc-metallopeptidase. It is present in human blood and its activity increases with advancing gestation reaching approximately threefold at term of delivery compared with nonpregnant women [46].

Mass production of a recombinant soluble form of APA (rec APA) using a mammalian cell-culture system, which enabled us to investigate APA *in vivo* for antihypertensive drug potential, has been successful. The cultured medium of 650 ml yielded 1.46 mg of soluble rec APA [47]. This system yielded production of rec APA in the order of milligrams per liter of cultured medium. Considering very low therapeutic doses for antihypertensive efficacy of rec APA *in vivo*, which will be described below, production of rec APA can be scaled up to a commercial level on a relatively compact capacity.

Bolus intravenous injections of rec APA at the dose of 0.016 mg/kg significantly decreased systolic BP in SHRs (approximately 165 mmHg). After 3 h it caused a decrease of BP by approximately -30 mmHg. It continued to cause a decrease in systolic BP, reaching approximately -40 mmHg after 24 h. Although its effect on BP in SHRs at the doses of 0.8, 0.16, and 0.016 mg/kg (n = 5 per doses) were examined, the lowest dose of 0.016 mg/kg presented a steady decline in systolic BP equivalent to the 0.16 mg/kg dose.

The 0.016 mg/kg dose of APA failed to influence systolic BP at 48 h in WKY rats. The antihypertensive effects of daily bolus injections of APA (0.016 mg/kg) against continuous infusion of the ARB candesartan (0.1 mg/kg/day) over 5 days in SHRs were compared. The magnitude of the antihypertensive effect by APA and by candesartan were similar: APA, 175.8 ± 13.5 mmHg to 147.6 ± 16.3 mmHg at 24 h; ARB, 169.5 ± 10.3 mmHg to 137.4 ± 11.0 mmHg at 24 h (**Figure 5**) [48].

Daily bolus administration of APA (0.016 mg/kg) or continuous infusion of candesartan (0.1 mg/kg) for 5 days indicated that the antihypertensive effect of APA was similar to that of candesartan. It is known that intravenous administration of the ARB Dup753 (10 mg/kg) decreases BP in SHRs, which continues for at least 24 h [49], and intravenous injection of the ACE inhibitor captopril (2 mg/kg) reduces BP in SHRs for up to 3 h [50]. The effective dose of APA is about one-tenth of that of candesartan. Thus, in a comparison of a bolus injection of APA (MW 109 kDa) and a continuous infusion of candesartan (MW 440 Da) for 36 h, APA possesses a 2300-fold efficacy over candesartan by molecule-to-molecule comparison. The antihypertensive effects induced by a bolus injection of APA are similar to those obtained during continuous drip infusion of nitroprusside (0.5 – 5 mg/kg/min) in humans (**Figure 5**) [51].

Recombinant APA to treat hypertension by degrading circulating A-II before binding to the receptor 1 subtype could be a novel approach for various hypertensive disorders, including hypertensive encephalopathy, apoplexy, acute heart failure and acute dissection of aorta, as well as preeclampsia.

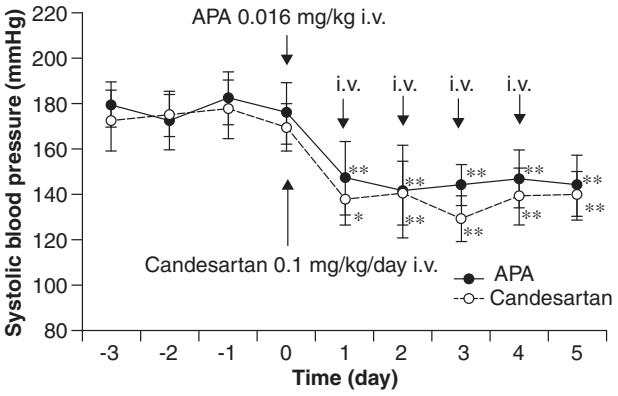

Figure 5. Comparison of APA (0.016 mg/kg) and candesartan on BP in SHRs. Intravenous injection of recombinant APA (n = 5) and candesartan (n = 5) significantly decreased systolic BP for 5 days in SHRs. The magnitude of the antihypertensive effects of APA (0.016 mg/ kg) and ARB (0.1 mg/ kg) was similar. APA (0.016 mg/kg): bolus injection every day for 5 days; candesartan (0.1 mg/kg): continuous infusion by osmotic mini-pumps for 5 days.
*p < 0.05, **p < 0.01 compared with pretreatment period.

7. APA is a potential candidate drug for hypertension in pregnancy

It is well known that administration of ACE inhibitors causes hypotension, which can be fatal to the human fetus. This suggests an important role for A-II in the feto-placental circulation [52]. Fetal circulation might be influenced by these peptide hormones, resulting in vasoconstriction. The fetus increases A-II and AVP under stress [5-7]; thus, the fetus is forced to increase BP in the feto-placental unit.

Fetal BP is much lower than the mother's and BP of placental peripheral circulation is lower than the fetal BP. When conventional antihypertensive agents are administered to preeclampsia patients, these chemicals easily diffuse to the feto-placental unit and then they can block the fetal response against hypoxia, an effort to increase BP.

Pregnant SHRs were used to identify an effective dose of rec APA for hypertension in pregnancy and to study the safety of rec APA on the pups in comparison with ARB [48]. The doses were as follows: recombinant APA (0.016 – 0.16 mg/ kg/day) (n = 5) and ARB (candesartan: 0.1 – 1 mg/kg/day) (n = 5). Initial doses of ARB were titrated to correspond with those used for human hypertension. The drugs were injected, and for both drugs each dose was doubled daily up to 10-fold until the end of the treatment (day 10 to day 20 of pregnancy). In the protocol, daily doses of rec APA and ARB were adjusted to correspond with the predicted estimated amount of fetal A-II, which will increase in proportion to the daily fetal growth.

Figure 6 shows changes in systolic BP during mid- and late-pregnancy in SHRs after administration of candesartan and APA. The magnitude of antihypertensive effects of APA and candesartan were similar at day 20 (-30 mmHg). Many mature renal glomeruli, which contain red blood corpuscles, were found in SHRs treated with APA, whereas only a few were identified in the SHRs treated by ARB. The dose-escalating protocol was based on previous findings including clinical observations in humans that the maternal serum concentrations of APA showed an increase at the onset of preeclampsia, and thereafter decreased as the disease condition was worsened and hypertension became severe [46,53]. The authors' hypothesis is that APA acts in opposition to increasing fetal A-II but this balance is altered in severe preeclampsia in deficiency of APA [54,55]. This represents increasing partial efflux of A-II from the feto-placental unit into maternal circulation, and this physiology occurs with advancing gestation in normal pregnancy, but also exists in preeclampsia (**Figure 1**). If APA were used in future for treating preeclampsia, daily doses of rec APA should be increased to match the estimated increase of fetal A-II levels.

Although a number of pathogenic factors have been proposed to underlie the hypertension of SHRs [56], the authors' study suggests an important role for APA-mediated degradation of A-II in pregnant SHRs [48] as well as in nonpregnant SHRs [3]. The molecule size of APA is large enough for it to not pass through the placenta. Therefore, APA can be a useful and safe drug for the treatment of preeclampsia.

8. P-LAP is a potential candidate drug for pregnant uterine contraction

Insulin-regulated aminopeptidase [57] is the rat homolog of P-LAP [58]. Keller *et al.* reported that the P-LAP KO mice did not have problems in maintaining pregnancies and did not deliver their pups prematurely [59]. In contrast, as mentioned earlier, P-LAP KO mice did deliver their pups prematurely and showed increased sensitivity to exogenously administered OT [27]. These data suggest the importance of balance between OT and its degradation by P-LAP in both preterm labor and onset of spontaneous labor.

Daily maternal serum P-LAP activity increases progressively during late pregnancy, reaching a relatively high level at 11 days prior to the onset of labor, then fluctuates slightly until the onset of labor [60]. This observation suggests an increased sensitivity to exogenously administered OT just prior to the onset of labor. As P-LAP activity levels off, the clearance of OT will be deceased. This means at this stage, pregnant uteri seem to have become highly sensitive to fetal OT [9].

The activities of P-LAP in serum obtained from normal nonpregnant and 1536 pregnant women were used for normal P-LAP range with advancing gestation. The serum P-LAP of patients who were admitted to the hospital due to preterm labor was compared with normal reference values [61]. Of 61 patients, 28 patients had preterm deliveries (preterm group) and the remaining 33 patients delivered in normal

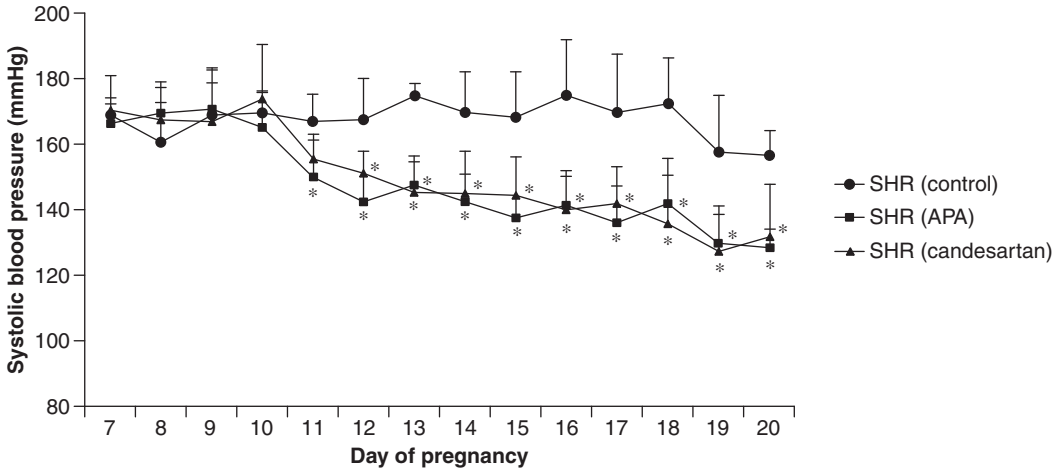

Figure 6. The changes of systolic BP during mid- and late-term pregnancy in SHRs (control, n = 5), with administration of SHR (APA: 0.016 – 0.16 mg/kg/day, n = 5) and SHR (candesartan: 0.1 – 1 mg/kg/day, n = 5). APA and candesartan decreased BP of pregnant SHRs.
*p < 0.05 compared with pretreatment period.

term (normal term group). Women admitted to the hospital from threatened preterm labor were treated with conventional medications (β-agonists, magnesium sulfate, or any other tocolytics, alone or in combination, and/or bed rest). Labor occurred at week 38.7 ± 1.2 in the term group, whereas it occurred at week 33.3 ± 2.4 in the preterm group.

Patients with serum P-LAP activities below the 10th percentile had a greater ratio of preterm delivery than the term group (p = 0.0085) and approximately 2.3-fold increase in the risk of preterm delivery. In this study, P-LAP activity was assayed only once at admission (around week 31). All patients had regular uterine contractions at < 15-min intervals. High specificity (95%) of this test suggests a direct role of the OT/P-LAP system in preterm labor. These laboratory and clinical data suggest that the OT/P-LAP system was functioning at the onset of normal labor and preterm labor.

A mass production system for the recombinant soluble form of P-LAP (rec P-LAP) using Chinese hamster ovary cells was established [62]. The effects of rec P-LAP on pregnant mice were examined. The administration of rec P-LAP at 0.1 U/day continuously from day 15.5 in WT mice resulted in the prolonged onset of labor by 2 days or more without any negative effects on both the animal and pups. Administration of recombinant P-LAP at the dose 0.01 U/day continuously from day 15.5 in WT mice resulted in a significant delay of the onset of labor by 1.5 days compared with the control. The daily dose of 0.01 U of rec P-LAP is expected to prolong the onset of labor by approximately 1 day.

The adenylate cyclase activator, forskolin, has been shown to upregulate P-LAP gene expression [63-65]. During the early stages of both preeclampsia and preterm labor, the fetus becomes hypoxic resulting in an increase of AVP [7]. AVP enhances the intracellular cAMP levels via its receptors [66]; thus, increased AVP may lead to induction of P-LAP expression in trophoblasts. Increased AVP may initially upregulate P-LAP levels, which counteracts hypertensive and uterotonic activities of AVP. As preeclampsia and preterm labor worsen, further incremental P-LAP activities do not occur. Recently, the authors found that pregnant P-LAP KO mice were significantly hypertensive throughout pregnancy compared with the WT pregnant mice.

Johnson *et al.* [67] identified two novel quantitative trait loci for preeclampsia susceptibility on chromosomes 5q and 13q. They concluded that, in preeclampsia, genes encoding aminopeptidase enzymes are P-LAP and adipocyte-derived leucine aminopeptidase, both of which belong to the P-LAP subfamily [68].

In light of unavailability of appropriate treatments for preeclampsia and preterm labor, rec APA and rec P-LAP could be possible candidates for these diseases. It is hoped that pharmaceutical companies commit to develop these candidates.

9. Conclusion

Rec APA acts as an antihypertensive agent. The effective dose of rec APA to achieve significant reduction of BP in SHRs is approximately one-tenth of the effective dose of candesartan. To degrade circulating A-II by rec APA before binding to the receptor 1 subtype is considered a novel approach for various hypertensive disorders including preeclampsia. The administration of recP-LAP at the dose of ≤ 0.1 U per day continued from day 15.5 in WT mice resulted in the prolongation of the onset of labor for one or more days without any adverse effects on both pregnant WT mice and pups. Rec APA and P-LAP could be promising candidates.

10. Expert opinion

Since 1970, basic and clinical studies to reveal the mechanisms underlying preeclampsia and preterm labor have been studied. The importance of the role of placental aminopeptidases in both conditions has been clarified. This is based on the findings of studies on dynamics of peptide hormones secreted by the fetus, namely, A-II, AVP, and OT. The results of clinical studies suggest critical involvement of placental aminopeptidases in preeclampsia and preterm labor. Therefore, recombinant technologies were employed for the mass production of the soluble rec-aminopeptidases, rec APA and P-LAP. They were used for the preclinical studies that followed. Thereafter, it has been suggested that both rec APA and rec P-LAP can be effective and safe treatments for preeclampsia and preterm labor, respectively. There was no evidence-based maternal- and fetal-safety medical strategy available in this area until recently. To progress with this innovative challenge of developing drugs indicated for incurable diseases, medical professionals need to cooperate with drug companies willing to invest.

ACE2 added complexity to the RAS. There is an opposite view on the involvement of ACE2 in the RAS in humans. The antihypertensive effects induced by rec APA are similar to those induced by nitroprusside infusion in humans. Rec APA can potentially reverse various hypertensive crises, such as hypertensive encephalopathy, apoplexy, acute heart failure, and acute dissection of the aorta. Kaplan mentioned that A-II is inactivated by a series of aminopeptidases, angiotensinase, present in most tissues and in very high concentration in red blood cells [69]. As APA actively destroys A-II in circulating blood and prevents it binding to A-II receptors. In the series of aminopeptidases, the most important one that was suggested by Kaplan was APA.

APA is responsible for hypertension in SHRs. It is an intrinsic enzyme that antagonizes hypertension similar to the mechanism seen with insulin in diabetes mellitus. P-LAP should provide us with the answer for the longstanding enigma of human labor.

If the new drugs mentioned here were available, preeclampsia and preterm labor might be cured. There is also hope that the number of premature babies and heart transplantations in children, as well as suicide in young male adults, might be decreased.

The new drugs proposed here are showing new aspects of the treatment of preeclampsia and preterm labor. As far as we know, only ARB and ACE inhibitors are effective antihypertensive drugs for preeclampsia [3]; however, they are prohibited during pregnancy. APA might be the most probable candidate drug to replace them. Among the causes cited for the "premature labor syndrome" are preterm uterine contractions, residual activation, as well as cervical incompetence [69]. Based on current knowledge, these processes are receptor mediated, with a central role being ascribed to the OT/OT receptor system, particularly with regard to uterine contractions as well as residual activation [70]. Uterine contraction in preterm labor is controlled with the balance of fetal AVP, OT, and P-LAP; therefore, the OT receptor antagonist, atosiban, is the most reasonable drug for treatment of preterm labor [71]. However, P-LAP could be a promising candidate and superior to atosiban as it does not pass through the placenta. Both APA and P-LAP lyse peptide hormones while in vascular circulation and prevent their biological actions (vasoconstriction and uterine contraction) before binding to the receptors.

Since 2012, tissue engineering technology with induced pluripotent stem (iPS) has been gaining public attention. A tremendous amount of the research budget, not only from pharmaceutical companies, but also by the government, has been invested in this area. It is imperative to invest in obstetrics for safe and effective medications. Development of new drugs in obstetrics is very important compared with restoring damaged organs in the elderly.

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