

## NPO Society For Caring Fetus and Mother In Pregnancy Induced Hypertension And Preterm Labor (P-LAP Society)

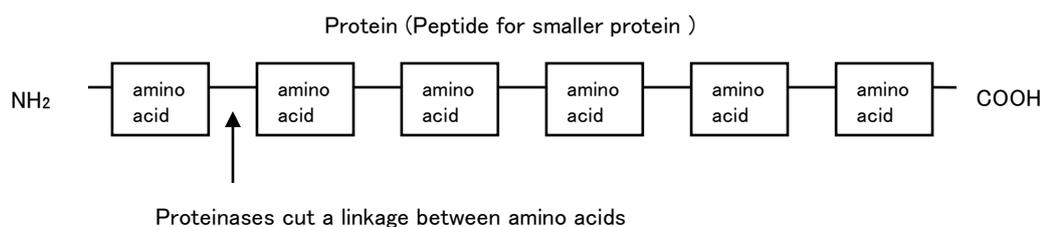
A memorandum for placental peptidases and estrogen-progesterone therapy for pregnancy induced hypertension and preterm labor

### 1) The role of placental peptidase

What is placental peptidase ?

Human body is composed of the protein. Protein is taken as nutriment in the food. Proteinase is a substance that decomposes the protein in the body, in a broad term the enzyme. Placental proteinase is therefore, the enzyme secreted from the placenta which decomposes or lyses protein. This enzyme is also a kind of protein which lyses another specifically targeted protein called substrate.<sup>1</sup> The protein composes human body has various functions and it is a conjoint substance made of predisposed sequence of the amino acid.

There are 20 amino acids found in the food. Such as glutamine or aspartic acid are well known. Proteinase disconnects each linkage between the two amino acids, but in a sequential manner and on a precisely specific domain, thus, the substrate is decomposed into smaller pieces and their functions become completely different. Even now, new physiological functions of these small sized proteins, called the peptide, is discovered through biological research. The final piece of decomposed proteins or peptides is a single structure of amino acid.



Various kinds of hormones are secreted from the fetus and the placenta when the mother became pregnant. These hormones are the protein consists of a number of amino acids. The enzymes decompose peptides are called peptidases, which are produced in the body and are able to exert their functions with extremely small amounts of molecules. Unlike synthesized chemicals different structures from human origin substances, these peptidases or proteinases are natural biological substances, therefore they are deemed safe for both mother and fetus compared to those extrinsic chemicals. Some of important examples are oxytocinase which lyses oxytocin and angiotensinase which lyses angiotensin II. These are explained in details in the pages that follow.

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<sup>1</sup> Protease is the enzyme which decomposes protein. peptidase is the enzyme which decomposes small sized protein called peptide. Oxytocinase decomposes hormone named oxytocin responsible in inducing labor by muscle contraction in the uterus.

The next graph (graph 1) shows the concentration of placental proteinase in the maternal blood circulation periodically measured in the course of gestational period. The placental proteinase is elaborated in the placenta<sup>2</sup> and effuse into the maternal circulation. As the gestation progresses, its concentration gradually increases during early phase and a rapid increase observed at around weeks of 20<sup>th</sup> to 30<sup>th</sup>. Serum concentration can be measured in the maternal circulation,<sup>3</sup> which means the proteinase can exert effects on the mother's physiology. The important function of placental proteinase is the regulation of hormones between the fetus and the mother.

Graph 1

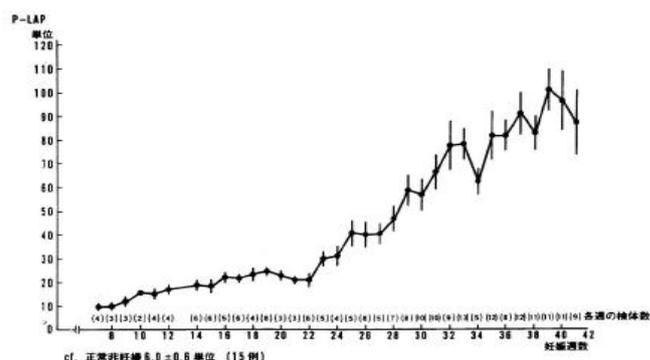


図 1. 正常妊娠における P-LAP の動態 (19 例, 234 検体, 平均値±標準誤差)

Graph 1 shows maternal blood concentration of placental proteinase by gestation week. By looking at fluctuations, prognosis of normal or abnormal gestation can be predicted. It is an important surrogate marker for pregnancy induced hypertension and the risk of preterm labor. The scientific terminology of the proteinase is placental leucine amino peptidase or P-LAP. Oxytocinase is one of the P-LAP family.

For a good healthcare for women and her baby during gestation, adequate amount of exercise, sleep, balanced diet, and most importantly, avoid the stress. The adverse effects of stress to the fetus cannot be underestimated. Next we look at how the placental proteinase reacts against the stress comes from the mother's body.

The fetus secretes various hormones during growth. These hormones are small sized proteins ie., peptides. When the fetus is exposed to the stress, increased amount of peptides are secreted in order to safeguard itself against stress, for example, the fetus will need an elevation of blood pressure and heart rates which calls for increase of angiotensin, a peptide brings contraction of the blood vessels<sup>4</sup>(vasocontraction)

Increased secretion of angiotensin protects fetal circulation but at the same time, it exerts effects on the maternal blood pressure because the excess amount of angiotensin can flow

<sup>2</sup> Later in the research, identical proteinase was found in the tissues of pancreas, skeletal muscles, fat tissue, and in brain cells.

<sup>3</sup> In the assay, L-methionin is added to inactivate all other proteinases except placental origin. Ref; Mizutani.S, Noto.H,Inamoto.Y,Sakura.H,Kawashima.Y. Estimation of placental leucie aminopeptidase in abnormal pregnancy sera. Acta Obst Gynec Jpn vol.31,no.4, April 1979

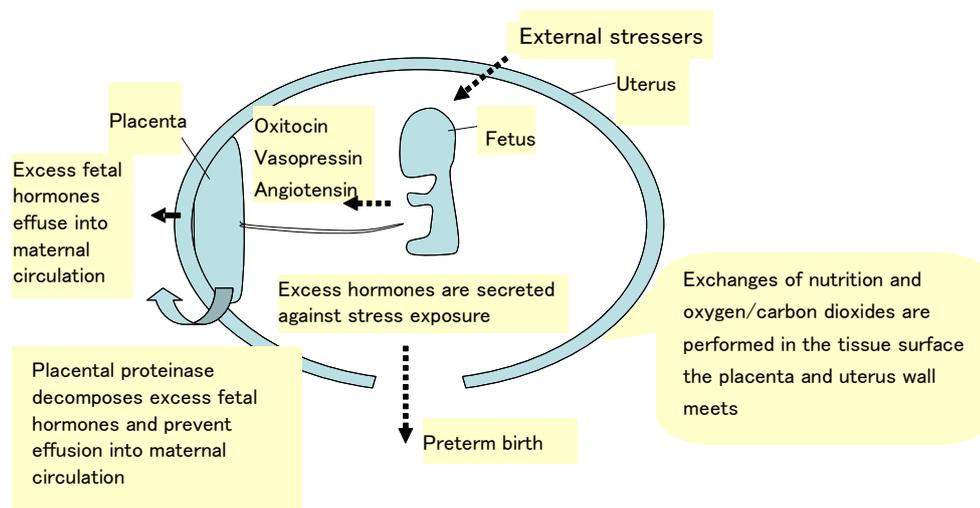
<sup>4</sup> Angiotensin: Angiotensin II is a peptide composed of 8 units of amino acids, elaborated in the liver. This peptide possesses a potent vasocontraction property. An inhibitor of angiotensin converting enzyme (ACE) has become a common anti-hypertensive drug but it has teratogenic risks on the fetus. Placental proteinase cuts one unit of angiotensin II amino acid chain and convert it to angiotensin III, the potency reduced by 80%.

into mother's circulation if it is not controlled. Deficiency of placental proteinase can lead to this imbalanced situation. Heavier deficiency should allow higher amount of penetration, thereby pregnancy induced hypertension of the mother may be induced. It is important to be aware of that the background of pregnancy induced hypertension may have been fetal stress exposure.

There are many other fetal peptides, for example oxytocin a well known peptide essential for the growth of the fetus. The amount of oxytocin increases in the course of fetal growth. If the placental proteinase were insufficient to correspond the increased oxytocin, the mother can be affected by excess oxytocin in the mother's circulation. If the fetus suffer from stress, a different kind of peptide hormone called vasopressin will be secreted and its amount may increase against heavy stress. Here again, insufficient placental proteinase causes imbalanced flow of the peptide allowing fetal peptide hormones to effuse excessively into the maternal circulation.

Oxytocin has its function to stimulate muscle contraction of the uterus. Therefore, high amount of oxytocin in the mother's body may cause expulsion of the fetus. The fetus will then be free from the stress but at the big cost of preterm birth. At the end phase of gestation, the secretion balance between oxytocin and placental proteinases will be physiologically collapsed bringing onset of spontaneous labor.

An illustration for Uterus, Placenta and Fetus



Maternal stressors directly affect the fetus, then fetus starts reacting against stress by secreting peptide hormones such as angiotensin, vasopressin, and oxytocin, the former two reinforce fetal circulation corresponding hypoxic utero-environment. These peptide hormones are inactivated by placental proteinases in a normal physiological balance and suppress excessive effusion into the maternal circulation. If excessive peptides are circulated, they can exert pathological effects such as hypertension or preterm labor. Angiotensin II possesses effects on vasoconstriction, vasopresion on fluid reabsorption and vasoconstriction, oxytocin on uterocontriction. Other than environmental stressors, genetic factors, mothers age, underlying complications, alcohol intake, smoking, environmental chemicals are all potential risk factors for above conditions.<sup>5</sup>

<sup>5</sup> Basic science and clinical application of aminopeptidases –from reproduction to malignant tumor- Edited by Mizutani.S. Medical Sense 2004

## 2 ) Relations between placental proteinase and female hormones, estrogen and progesterone

### Estrogen and progesterone

Two important female sex hormones namely, estrogen and progesterone are secreted from the follicle and corpus luteum (post ovulation tissue) respectively. Progesterone is imperative for establishment and maintenance of pregnancy. The fertilized ovum attaches (implantation) to the membrane wall of the uterus, then the pregnancy is determined completed. Estrogen and progesterone stimulate placenta and enhances secretion of placental proteinases. Therefore, placental proteinases can be supplemented by administration of these two female sex hormones.

The pharmaceutical estrogen and progesterone currently used are synthesized by a modern technology without any impure substances. Their chemical structure are therefore, identical to the human natural hormones and work in the same manner as human hormones in the human body. Because of this reason, administration of these synthesized hormones are safe, provided, the correct dose is used. Needless to say the one important matter is safety to the fetus. Estrogen and progesterone interact with placenta and stimulate production of placental proteinases, but not transmissible to the fetus.

### Treatment for pregnancy induced hypertension

Many drugs currently used for treating pregnancy induced hypertension are small in the size of the molecule which can easily penetrated into the fetal circulation. Since the blood pressure of the fetus is just about 40mmHg, antihypertensive drugs can invade easily and affect the fetus adversely, even if these drugs may be effective to treat the mother. The fetus under the stress requires to maintain its blood pressure and heart rates for protecting itself.

What is deemed worse for the fetal health is a certain type of the drug called beta adrenal receptor stimulants,<sup>6</sup> These beta stimulants, can alter activity level and expression of the beta receptors if the fetus were exposed long term under high doses of the drug. Because the speed of fetal development is extremely fast, drug exposure on the brain receptors can cause sensitivity imbalance between sympathetic and parasympathetic nerves, eventually may give rise to psychological impairment as the infant grow older.<sup>7</sup> The treatment of the mother has to be carefully done not to impose effects on the fetus being under delicate stages of the development of its life.

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<sup>6</sup> Beta stimulants: The body tissue has hormone receptors differentiated by the subunits type alpha( $\alpha$ ), beta( $\beta$ ), and gamma( $\gamma$ ). beta stimulants are pharmaceutical compounds which selectively bind to type beta2 receptors and exert a vasodilation effect. Originally for the treatment of asthma, but sometimes used for suppressing muscle contraction of uterus for preventing preterm labor.

<sup>7</sup> FR Witter et al. In utero beta2 adrenergic agonist exposure and adverse neurophysiologic and behavioral outcomes. American Journal of Obstetric & Gynecology Dec. 2009

### 3) Effectiveness, safety and the limit of estrogen-progesterone therapy by clinical cases

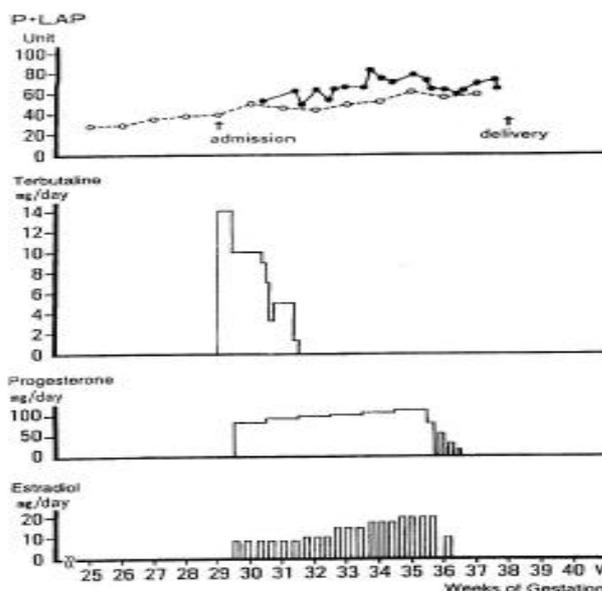
#### a) Clinical cases switched from beta stimulants to estrogen-progesterone therapy

##### Case 1 <sup>8</sup>

26 years old, nulligravida, with chief complaints of repeated pain in the uterus. With the sign of dilatation of cervix, she was admitted at 28<sup>th</sup> gestational week. terbutaline (beta-stimulant) was administered in dose escalation. Because the patient incurred in arrhythmia after continued palpitation, estrogen-progesterone therapy was initiated at the week 29<sup>th</sup> followed by dose increase until the week 34<sup>th</sup>. Observation at this point necessitated swift dose reduction of terbutaline in opposed to alleviated symptoms of preterm delivery. Placental proteinase started to rise as shown in the chart (upper chart, closed circle) During weeks of 34<sup>th</sup> and 35<sup>th</sup>, the dose of estrogen-progesterone was lowered. At the week 37<sup>th</sup>, the labor started and a female newborn weighed 3210 grams was uneventfully and spontaneously delivered.

Chart 1

Movement of placental proteinase concentrati



Movement of placental proteinase shown in the graph with closed circles. Gradual increase is observed after the administration of estrogen-progesterone therapy.

Betastimulant, terbutaline was withdrawn after dose reduction

Daily administration of progesterone continued until the week 36<sup>th</sup>

Estrogen was administered every other day

##### Case 2 <sup>8</sup>

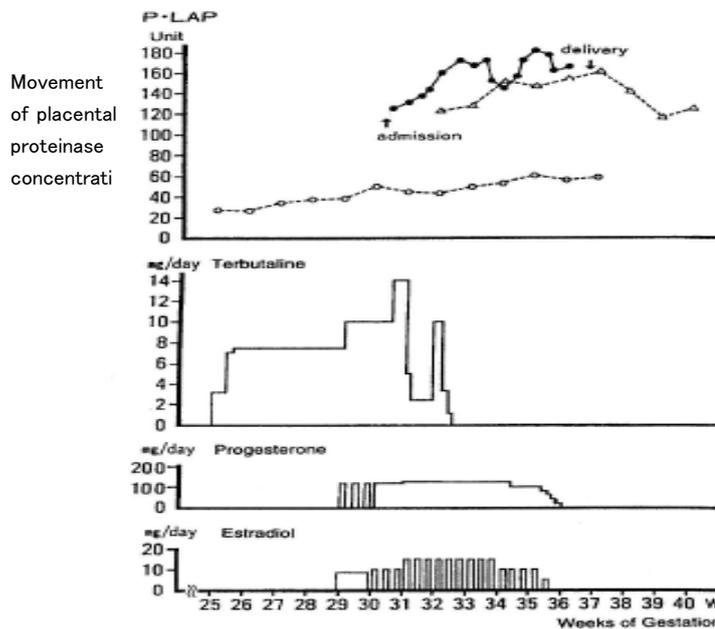
36 years old, gravida 4, parous 1, Received Shirodkar operation (sutures for tightening loose cervix) at week 15<sup>th</sup> due to the twin. She admitted another hospital complaining repeated uterus pain. Terbutaline was administered at the week 25<sup>th</sup>, however alleviation of the symptoms could not met. At the week 29<sup>th</sup>, estrogen-progesterone therapy was dictated. She was admitted to our hospital at the week 30<sup>th</sup>, and terbutaline dose was reduced until withdrawal at the week 33<sup>rd</sup>.

Estrogen-progesterone therapy was continued until the week 36<sup>th</sup>, placental proteinase increased gradually reaching the normal level or above that of twin pregnancy. As indicated in this case, the level of placental proteinase should keep approximately 50%

<sup>8</sup> Naruki.M, Mizutani.S, Yamada.R, Itakura.A, Kurauchi.O, Kikkawa.F, Tomoda.Y. Changes in maternal serum oxytocinase activities in preterm labor. Medical Science Research. 1995; 23,797-802

higher than the level of singleton. This is shown by the graph with closed circles. The graph with open triangles is the average of twin normal cases shown for comparison. The lower two graphs shows administration patterns of estrogen-progesterone therapy by concentration of progesterone (third graph) and estradiol. (the most common type of the chemical structure of estrogen hormones in the body) (fourth graph) Continuous line of the graph indicates daily administration of hormone injections and bar graph indicates intermittent administration (every other day), both intramuscular injections. The same drug but the method of administration may differ. At the week 37<sup>th</sup>, the labor started and female twin weighed 2415 grams and 2660 grams were uneventfully delivered.

Chart 2



Movement of placental proteinase indicated by the graph with closed circles. An increase is observed after estrogen-progesterone administration. Drop at the week 34<sup>th</sup>, and rebounded after.

Terbutaline, a beta stimulant, was reduced in dose after admittance then withdrew

Administration of progesterone started on intermittence thereafter switched to continuous injections

Administration of estrogen is continuous

Case 3 <sup>9</sup>

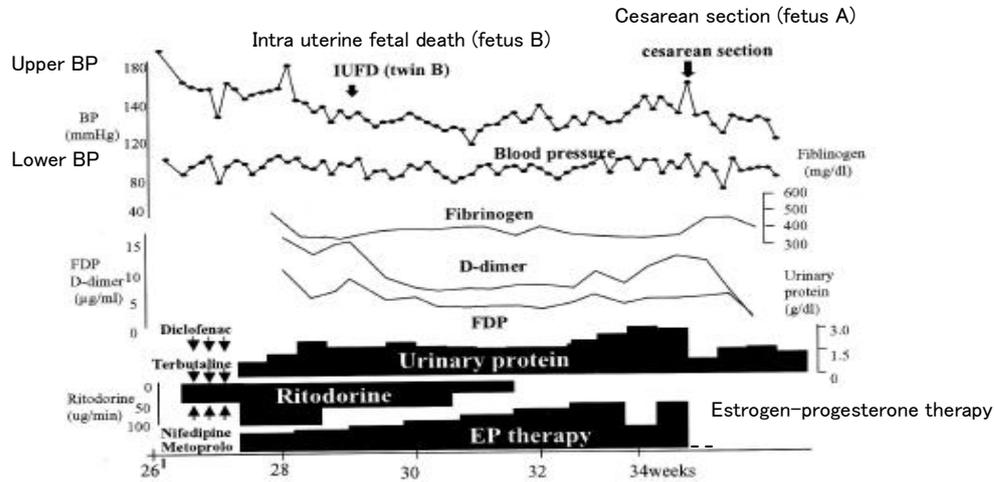
39 years old, diagnosed endometriosis and ovulation impairment. A twin pregnancy was established after IVF treatment. At the week 27<sup>th</sup>, she was admitted due to abrupt high blood pressure, diagnosed severe pregnancy induced hypertension. The blood pressure readings were 168 and 86 mmHg. Artificial fetus removal can be applied to restore immediately mother's blood pressure to the normal level, however, the family's preference to have two babies as healthy as possible, meaning gestation must be continued longer, the treatment to sustain pregnancy until targeted term without harmful effects to the fetuses was attempted.

Treatment initiated with diet regimen and rest cure. Ritodrine hydrochloride (beta-stimulant) as a base drug and estrogen-progesterone therapy was added in the middle course.(chart 3-1 EP) In order to minimize adverse effects of a long administration of beta stimulant, the dose was reduced until withdrawal at the week 31<sup>st</sup>. On the 3<sup>rd</sup> day of that week, bradycardia was observed on the fetus B thereafter confirmed stillbirth on the 4<sup>th</sup> day of the same week. Only estrogen-progesterone therapy was continued until the

<sup>9</sup> Takeuchi.M,Itakura.A, Terauchi.M, Sumigama.S, Mizutani.S. Case Repots of preeclampsia treated by 17-β estradiol propionate and hydroxyprogesterone caproate. (unpublished data)

week 34<sup>th</sup>. Prognosis was favorable with dose reduction. Recurrence of high blood pressure was noted on the 3<sup>rd</sup> day of the week 34<sup>th</sup>. Due to the detected increase of proteinuria, caesarian section was applied on the 5<sup>th</sup> day of the week 34<sup>th</sup>, and a female infant weighed 1684 grams was delivered uneventfully.

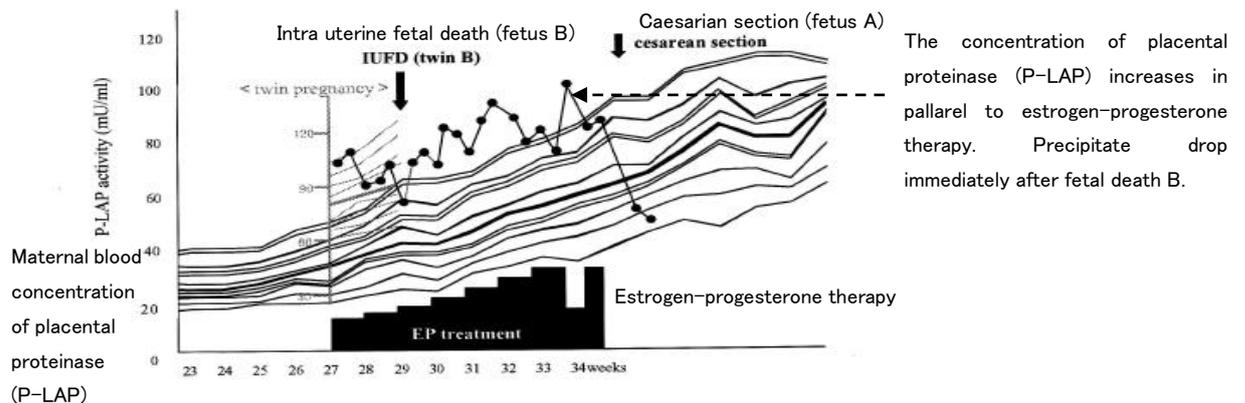
Chart 3-1



Nifedipine is an old commonly prescribed anti-hypertensive agent characterized by suppressing cell calcium inflow at muscle contraction. Fibrinogen is a precursor of fibrin fiber in the blood clot. D Dimer refers to fragments of fibrin clot. Hyper coagulability in the late phase of gestation necessitates monitoring of blood clot which may be induced by severe preeclampsia.

Chart 3-1 shows upper and lower readings of the blood pressure and their movement. About one month after initiation of the treatment, blood pressure stabilized to the normal level. The dose of ritodrine thereafter reduced while the dose of estrogen-progesterone was increased stepwise. The normal blood pressure was maintained until the week 34<sup>th</sup>.

Chart 3-2



<sup>10</sup> Ritodrine hydrochloride can be used under health insurance treatment for threatened preterm labor, however, this drug has adverse effects such as tachycardia, palpitation, moreover, requires dose escalation sometimes up to the maximum level. This is due to drug resistance and diminishing effects. Sometimes encounter the cases in which the maximum dose cannot suppress the symptom or the patient cannot tolerate.

Chart 3-2 shows movement of placental proteinase concentration in the maternal circulation. The effect of dose escalating estrogen-progesterone regimen explicitly indicates increasing the concentration of placental proteinase after concomitant administration of ritodrine hydrochloride on a dose lowering manner.

Effects of estrogen-progesterone therapy did not last long. At the week of 35<sup>th</sup>, the level of placental proteinase sharply decreased. The prolongation of gestation was achieved until 35<sup>th</sup> week and the risk of delivering an ultra-low birth weight infant was avoided. The girl born in this case grew healthy without sequela. This case shows the importance of extending gestation without using drugs that can pass placental barrier and exert adverse effects to the fetus.

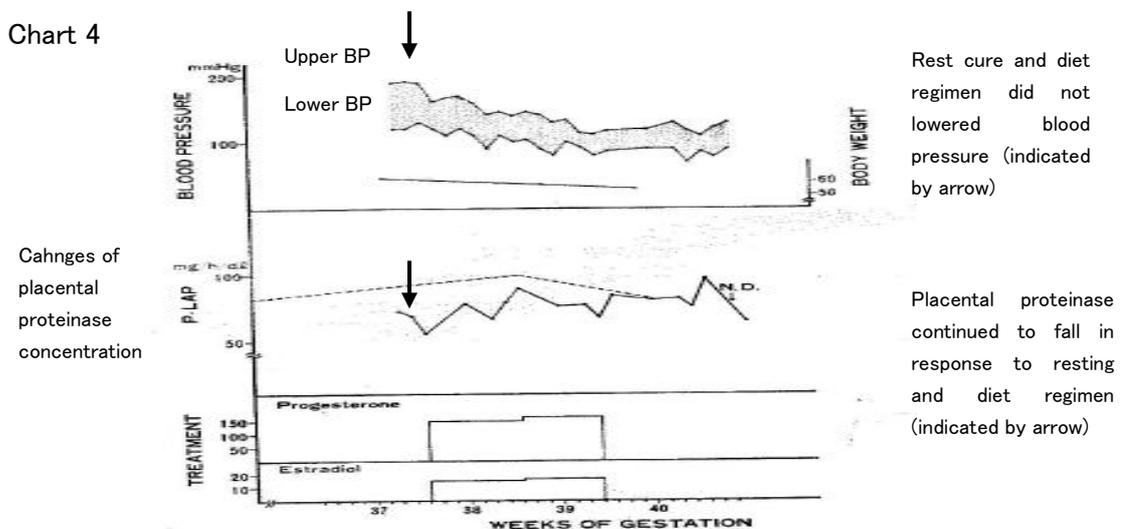
b) Clinical cases using estrogen-progesterone therapy only

Case 4 <sup>11</sup>

23 years old, primipara, unremarkable medical history. Mild proteinuria and oedema at the week 36<sup>th</sup>. Abrupt elevation of blood pressure at the week 38<sup>th</sup>, reading 170 and 110 mmHg, necessitated admission. Diet regimen and rest cure until day 5<sup>th</sup>, when the blood pressure rose to 190 and 130 mmHg and placental proeinase fell to 55.2mg/hour/dl. Estrogen-progesterone therapy was initiated immediately with a two-step dose escalation. On 8<sup>th</sup> day after the therapy, placental proteinase elevated to 90.6mg/hour/dl, thereafter the blood pressure normalized to 110 and 80mmgHg. At this point, estrogen-progesterone therapy was suspended and monitor clinical course. Gestation continued uneventfully until the week 41<sup>st</sup>, when the concentration of placental proteinase read 97.6mg/hour/dl at maximum, thereafter a male infant weighed 2500 grams was spontaneously delivered.

The weeks of 38<sup>th</sup> to 39<sup>th</sup> where one week of diet and rest cure without medication gave an important picture of changes of circulated placental proteinase, which was shown in the Chart 4. Placental proteinase gradually decreased and simultaneously blood pressure increased slowly. At this point of observation dictated the timing for initiating estrogen-progesterone therapy.

Chart 4



<sup>11</sup> Mizutani.S, et al. Steroidal treatment for pregnancy induced hypertension. Journal of Neonatology. No14 (4) 1978

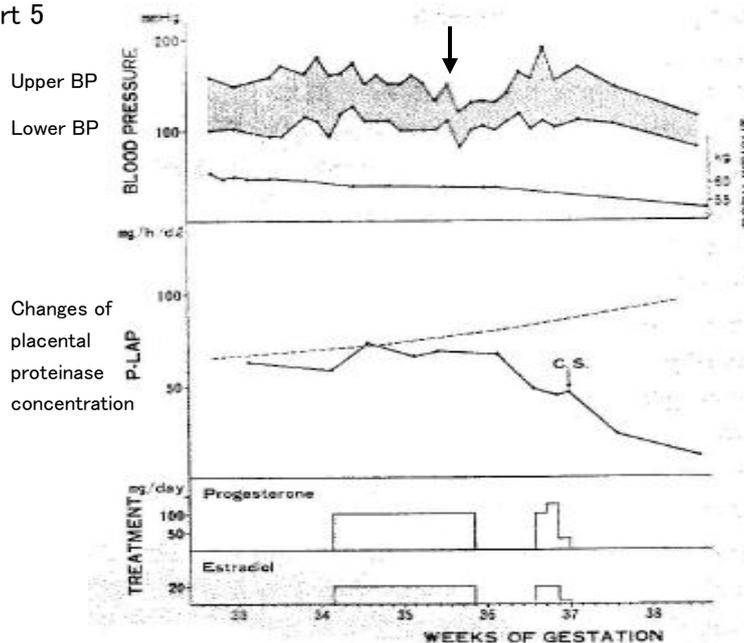
The effect of estrogen-progesterone on placenta was explicitly shown in this case. In relation to enhanced secretion of the placental proteinase, maternal blood pressure was steadily lowered. This indicates placental proteinase has decomposed excess amount of angiotensin II, a blood pressure elevating peptide, which effused into the maternal circulation. After the estrogen-progesterone therapy was withdrawn at the week 40<sup>th</sup>, blood pressure lowering effect was disappeared but the level of placental proteinase maintained to increase and eventually spontaneous delivery was witnessed. This case indicates maternal blood pressure can be effectively controlled with estrogen-progesterone therapy only. Prolongation of gestation until the week 41<sup>st</sup> at spontaneous delivery was uneventfully achieved.

Case 5 <sup>11</sup>

26 years old, unremarkable medical history, At the week 33<sup>rd</sup>, blood pressure rose to 158 and 100 mmHg, prominent oedema, proteinuria were recognized. The patient was admitted to the hospital. Diet regimen and rest cure continued until one week after admission. The blood pressure rose to 180 and 110 mmHg. At this point the estrogen-progesterone therapy was initiated. Similar to the previous Case 4, the starting time for the therapy can be determined by monitoring blood pressure and the concentration of placental proteinase during approximately one week of non-medicated regimen.

In parallel to the increase of placental proteinase, the blood pressure gradually decreased. At the week 36<sup>th</sup>, the blood pressure read 120 and 80 mmHg which was in the normal range. At this point, the therapy was stopped and observed recovery. The high blood pressure returned after the cessation of the therapy. Estrogen-progesterone was administered again, however, placental proteinase did not respond to rise. Caesarean section was applied and a female newborn was delivered weighed 1800 grams.

Chart 5



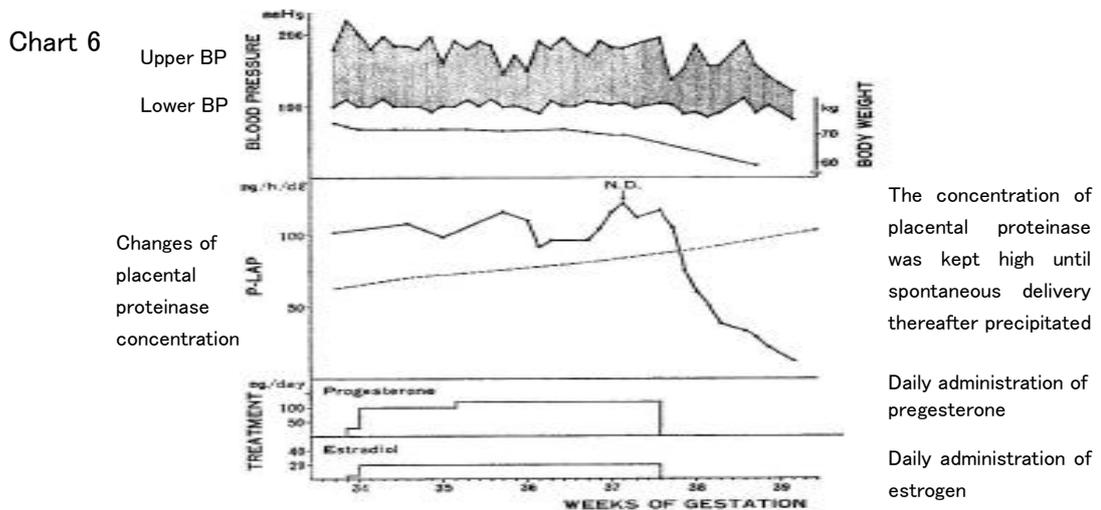
After cessation of estrogen-progesterone therapy, the blood pressure returned to normal. Placental proteinase fell after cessation of estrogen-progesterone therapy (BP rose in parallel to the fall of placental proteinase). Estrogen-progesterone therapy was suspended then restarted.

Estrogen-progesterone therapy initiated at the week 34<sup>th</sup> continued to the week 36<sup>th</sup> was effective in lowering blood pressure. Placental proteinase initially increased in response to the therapy. Thereafter this trend was diminished and started to fall after the week 36<sup>th</sup>. The blood pressure increased again despite re-administration of estrogen-progesterone. This case clearly indicates that the estrogen-progesterone therapy has a limitation in its duration of effectiveness to a certain period of time, which was in this case, three weeks. Monitoring of the maternal blood concentration of placental proteinase is therefore, important. Assay for placental proteinase is 80 points in the health insurance receipt in Japan but related kits in the market are not common.

Case 6 <sup>11</sup>

34 years old, parity 2, medical history of puerperal hypertension<sup>12</sup> immediately after delivery of the second baby. Since the week 27<sup>th</sup>, mild oedema was observed. At the week 31<sup>st</sup>, twin pregnancy was confirmed. From the week 34<sup>th</sup>, the blood pressure showed tendency to rise and the patient was admitted to the hospital. Estrogen-progesterone therapy was immediately started when the blood pressure read 220 and 110 mmHg. At the week 36<sup>th</sup>, placental proteinase moderately increased to 116.2 mg/hour/dl, and the blood pressure decreased to 142 and 92 mmHg. Concentration of placental proteinase increased to the maximum 122.6 mg/hour/dl, although blood pressure showed tendency of increase. At the week 38<sup>th</sup>, rupture of the membrane occurred. male newborn weighed 3000 grams and female newborn weighed 2360 grams were uneventfully delivered. The mother had puerperal eclampsia <sup>13</sup> 6 hours later and symptomatic treatments were given. The clinical course was uneventful and the patient was discharged later.

As shown in the Chart 6 third and fourth graphs, the combination dose seems to be optimal at the ratio of 1 for estrogen and 10 for progesterone per day.



<sup>12</sup> Puerperal is the term after birth until non-pregnant status, usually 6 to 8 weeks after. Puerperal hypertension onsets during this period.

<sup>13</sup> Eclampsia is the condition of systemic convulsion attack due to intracerebral oedema in abnormal circulation associated with hypertension. The precise cause is unknown.

Estrogen-progesterone therapy was initiated at the week 34<sup>th</sup>. Placental proteinase increased although some fluctuations were observed. The blood pressure also fluctuates but moves to decrease.

c) Severe clinical cases

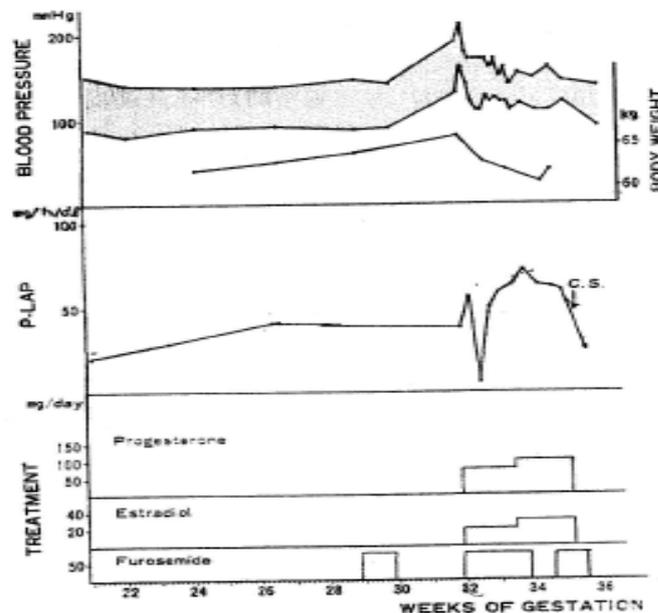
Case 7 (the first case with estrogen-progesterone therapy; 1970) <sup>11</sup>

29 years old, parity 1, medical history of severe pregnancy induced hypertension. Oedema from gestation week 29<sup>th</sup>, upper blood pressure rose to 190 mmHg at the week 32<sup>nd</sup>, with a strong positive proteinuria. The patient was admitted. Treatment initiated with antihypertensive diuretic, furosemide, to counteract precipitated rise of blood pressure.

The concentration of placental proteinase was low. Estrogen-progesterone therapy was initiated. Remarkable drop was observed then increased rapidly. Higher level of placental proteinase continued until the week 34<sup>th</sup>, then in the week 35<sup>th</sup> it started to drop despite continuation of the therapy. The blood pressure started to rise again and at this point caesarean section was applied. Male newborn weighed 1750 grams was delivered uneventfully.

Chart 7  
Upper BP  
Lower BP

Movement of placental proteinase concentration



Concentration of placental proteinase and blood pressure readings are counter-related each other

Placental proteinase showed precipitated fall thereafter recovered rapidly

Daily administration of progesterone

Daily administration of estrogen (estriol)

furosemide (diuretic)

After starting estrogen-progesterone therapy, the blood pressure was lowered, however, the effect did not continue long. The concentration of placental proteinase showed unstable rise and fall phenomenon. Despite this inconsistency, prolongation of gestation was achieved for as long as three weeks owing to the effect of the remedy.

d) Clinical cases with complications

Case 8 <sup>9</sup>

26 years old, Type II diabetes mellitus, parity 1, on insulin regimen. After pregnancy, proteinuria appeared. A mild dilatation of cervix was detected at the week 29<sup>th</sup> and the patient was admitted immediately. Upper blood pressure read 155 mmHg, positive proteinuria worsened to 3+. The patient was diagnosed severe pregnancy induced hypertension and ritodrine hydrochloride (beta-stimulant), steroid, and estrogen-progesterone therapy were initiated while the patient is on diet regimen and rest cure. On the fourth day of the week 32<sup>nd</sup>, administration of ritodrine hydrochloride was stopped. Estrogen-progesterone therapy was continued. The blood pressure was kept at more or less close to the normal level. Proteinuria improved. Gestation maintained to the week 37<sup>th</sup>, at this point rupture of the membrane occurred and caesarean section was applied because of breech presentation was associated. A male newborn weighed 2388 grams was delivered uneventfully.

Chart 8-1

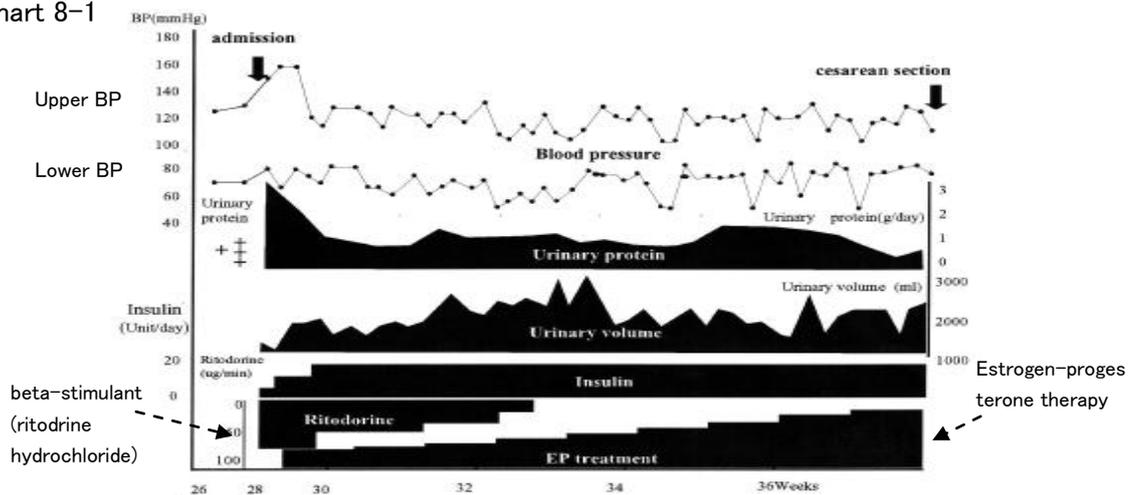


Chart 8-1 shows treatment course of estrogen-progesterone therapy. Along with the dose increase, the blood pressure comes close and kept to the normal level. The degree of proteinuria seems suppressed at the low level.

Chart 8-2

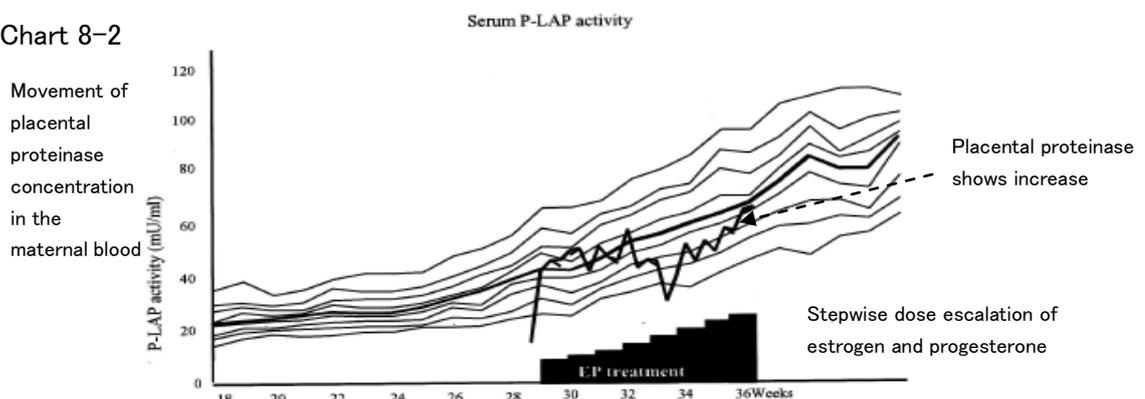


Chart 8-2 shows treatment course looked by placental proteinase concentration.(bold lone) A gradual increase of placental proteinase in response to dose escalation of estrogen and progesterone. This case elucidates the estrogen-progesterone therapy can be effective for extending gestation even in the presence of complications such as diabetes. The male newborn was delivered in a healthy condition and he is growing without sequela.

Case 9 <sup>8</sup>

31 years old, surgical history of endocardial cushion defect. Past history of intrauterine fetal death in 31<sup>st</sup> gestation week. Admission was commanded due to recurrent utero-contraction. Cardiac disorders such as in this case are contraindicated to beta stimulants, ritodrine hydrochloride for example. Estrogen-progesterone therapy that uses synthesized natural female hormones can be applied to these exceptional cases.

Estrogen-progesterone therapy was initiated at the week 21<sup>st</sup>. Placental proteinase gradually increased and the administration was withdrawn. The patient was discharged at the week 31<sup>st</sup>. The course afterward continued with a rise of normal placental proteinase concentration until the week 38<sup>th</sup>, and at this point male newborn was spontaneously delivered weighed 3090 grams.

Chart 9

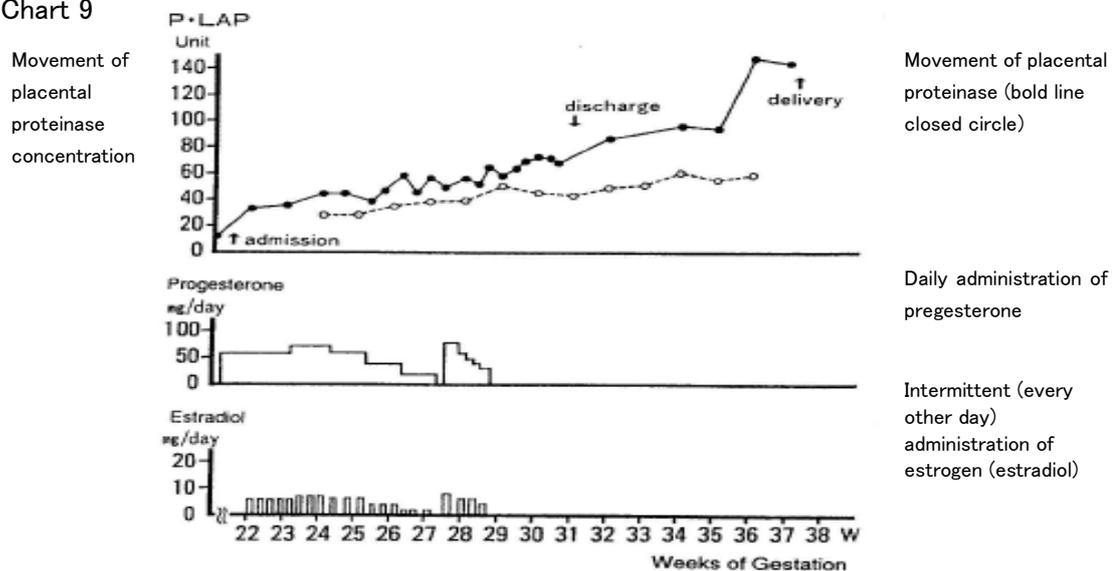
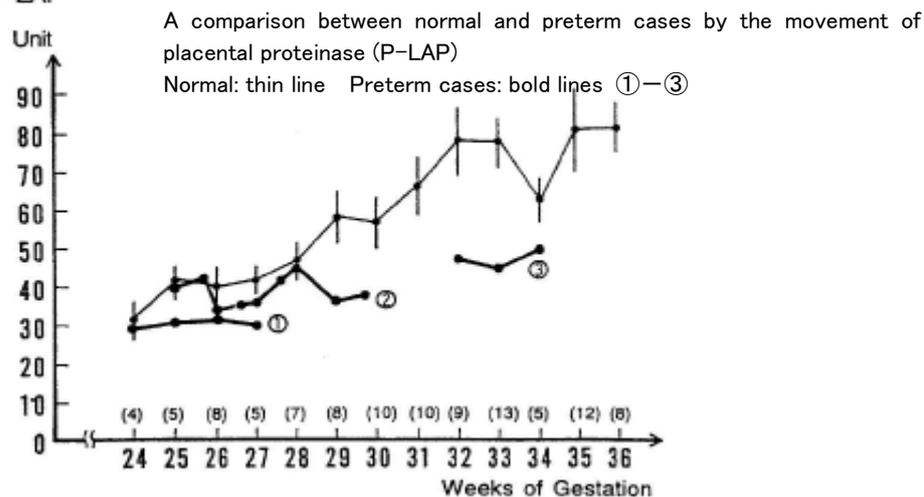


Chart 9 shows movement of placental proteinase in parallel to estrogen-progesterone therapy.(bold line closed circle) The therapy was initiated at the week 21<sup>st</sup> and the doses of both hormones were gradually lowered from the week 24<sup>th</sup>. The concentration of placental proteinase did not fall with lower doses. Three weeks before discharge, a short term administration of the estrogen and progesterone were given. The placental proteinase shows an increase in response. This increase becomes steep prior to the delivery, thereafter the normal delivery took place.

## Summary

Clinical cases shown in this report elucidate importance of monitoring changes of the placental proteinase concentration in the course of treating pregnancy induced hypertension and preventing preterm birth. The next chart 10 is given to show a summarized picture of three preterm cases in contrast to an average normal movement of the proteinase. The differences can be explicitly observed. <sup>8</sup>

Chart 10 P-LAP



Case① was a stillbirth 1750g at 27<sup>th</sup> gestation week, case② was premature birth 1430g at 29<sup>th</sup>, case③ was premature birth 1880g at 34<sup>th</sup> Cases ②③ are live births

## 4) Future of placental proteinase

The DNA sequence of the placental proteinase was discovered by a Japanese scientist whose name is doctor Shigehiko Mizutani MD, professor emeritus of Nagoya University department of obstetric and gynecology. His research goes back to 1996 when he published a literature regarding DNA sequence of oxytocinase, <sup>14</sup> one of the placental proteinase family, the existence of which was hypothesized in 1930s having an action to lyse oxytocin in labor regulation.

In parallel to doctor Mizutani's work, American researchers published a literature regarding the enzyme (proteinase) which co-locates with glucose transporter and moves together to the surface of the cell when stimulated by insulin. Later on this line of research, these two were found to be identical substances, however, the DNA sequence published by the American group later corrected their sequence data.

The placental proteinases are found in the cell membrane of the placenta and they are the protein consists of about 1000 pieces of amino acids with each end of molecule binds to one end to amide group and the other end to carboxyl group. There are a number of proteinases in this family. The critical ones related to pregnancy induced hypertension and preterm labor are oxytocinase and angiotensinase, the latter a specifically regulates

<sup>14</sup> Rogi.T, Tsujimoto.M, Mizutani.S. Tomoda.Y. Journal of Biochemistry 271.56-61 1996

blood pressure. Angiotensin is a small sized protein called peptide and it is a precursor of angiotensin II which is a powerful blood pressure elevating peptide. This metabolic process is mediated by one enzyme called angiotensin converting enzyme.(ACE) A commonly prescribed drug for treating hypertension in adults is an inhibitor of ACE, however, this drug can exert teratogenic effects on the fetus, therefore contraindicated in obstetrics.

Unlike ACE inhibitor, a synthesized chemical, angiotensinase is a larger sized protein biologically produced in the placenta with no harmful effects on the fetus. When the pregnant mother had increased amount of angiotensin II in her body tissue such as in the blood vessel, angiotensinase can decompose angiotensin II to angiotensin III which has about one-fifth potency of raising blood pressure. This rapid metabolic action driven by angiotensinase normalizes elevated maternal blood pressure while not affecting the fetus in maintaining adequate level of blood pressure.

Hence, treatment strategies for hypertension in adults and in the pregnant mother are completely different. This consideration necessitates every treatments applied to the pregnant mother should have a dual caution –mother and fetus–all the time.

As described earlier in this report, genetic structure of placental proteinase has been revealed by professor Mizutani and in his laboratory, recombinant placental proteinase was synthesized for experimental purposes. Many of its pharmaceutical functions were found by using this engineered compound. Therefore manufacturing pharmaceutical placental proteinase on a commercial scale should not be challenging.<sup>151617</sup> Let us hope this can be realized soon and eliminate risks off the mothers and infants.

##### 5) About NPO Society For Caring Fetus and Mother In Pregnancy Induced Hypertension And Preterm Labor (P-LAP society)

The P-LAP Society has three objectives, namely, provide public education with the knowledge of pregnancy induced hypertension and preterm delivery, promote estrogen–progesterone therapy for the treatment of these diseases, and establish a base for the development of pharmaceutical placental proteinase<sup>18</sup>

The members of the NPO will receive latest news regarding placental enzyme and related information globally in the column 「doctor Mizutani's lecture」. Those who are pregnant mothers and her families, who are involved in the womens' health in general may be interested in.

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<sup>15</sup> Mizutani.S et al. A new approach regarding the treatment of preeclampsia and preterm labor . Life Sciences 88(2011) 17-23

<sup>16</sup> Mizutani.S, Kobayashi.H, Etiological study of pregnancy induced hypertension and preterm labor and needs for developing a new treatment. Journal of JMA 139. 3.Jun.2010

<sup>17</sup> Mizutani.S. Physiological roles of placental leucine aminopeptidase/oxytocinase and its clinical application using molecular biological technique. Government science research fund no. 12470341 March 2003

<sup>18</sup> NPO prospectus: A society for caring mothers and neonates in pregnancy induced hypertension and preterm labor. March 2012

## Appendix

Treatment regimen for noncomplicated severe pregnancy induced hypertension using estrogen-progesterone mono therapy (for alleviating hypo placental-proteinemia) <sup>19</sup>

### I Protocol for singleton

Gestation week	Daily dose E2 (mg/dl)	Daily dose Progesterone (mg/dl)
20w	3.00 mg	60.0 mg
21w	3.25 mg	62.5 mg
22w	3.50 mg	65.0 mg
23w	3.75 mg	67.5 mg
24w	4.00 mg	70.0 mg
25w	4.25 mg	72.5 mg
26w	4.50 mg	75.0 mg
27w	4.75 mg	77.5 mg
28w	5.00 mg	80.0 mg
29w	5.25 mg	82.5 mg
30w	5.50 mg	85.0 mg
31w	5.75 mg	87.5 mg
32w	6.00 mg	90.0 mg
33w	6.25 mg	92.5 mg
34w	6.50 mg	95.0 mg
35w	6.75 mg	97.5 mg
36w	7.00 mg	100.0 mg
37w	7.25 mg	102.5 mg
38w	7.50 mg	105.0 mg
39w	7.75 mg	107.5 mg

Daily intramuscular injection after admission with low salt diet and rest cure

### II Protocol for twin pregnancy (Normal P-LAP concentration is x1.5 of singleton)

Gestation week	Daily dose E2 (mg/dl)	Daily dose of Progesterone (mg/dl)
27w	7.125 mg	116.25 mg
28w	7.5 mg	120.0 mg
29w	7.875 mg	123.75 mg
30w	8.25 mg	127.5 mg
31w	8.625 mg	131.25 mg
32w	9.0 mg	135.0 mg
33w	9.375 mg	138.75 mg
34w	9.75 mg	142.5 mg

<sup>19</sup> Mizutani.S. The protocol for progesterone replacement therapy in threatened preterm labor. Ngoya University Department of Obstetric and Gynecology 2000

Note:

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