Antithrombin and Preeclampsia
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ANTITHROMBIN AND PREECLAMPSIA

To understand the possible role of antithrombin (AT) in preeclampsia, it is important to understand (1) the pleiotropic effects of AT on the coagulation and inflammatory pathways and (2) the bases for the various manifestations of preeclampsia in pregnancy.

Antithrombin

Antithrombin is a complex glycoprotein with multiple pharmacologically important activities in both the coagulation and inflammatory cascades. Normal serum AT levels range between 12.5 – 15 mg/dL (Murano et al 1980). It is the most critical modulator of coagulation (Figure 1) and has potent anti-inflammatory properties (Figure 2) independent of its effects on coagulation (reviewed in Roemisch et al 2002).

Anticoagulant Properties of AT

AT is a serine protease inhibitor (Figure 1) that is the principal inhibitor of the blood coagulation serine proteases thrombin and Factor Xa, and to a lesser extent, Factors IXa, Xla, trypsin, plasmin, and kallikrein (Aubry & Bieth 1977, Lahiri et al 1976, Menache 1991, Menache et al 1992, Travis & Salvensen 1983). Binding of heparin to AT results in a conformational change that greatly increases the activity of AT toward thrombin (1000 fold) and other serine proteases. AT neutralizes the activity of thrombin as well as other serine proteases by forming a 1:1 stoichiometric complex between enzyme and inhibitor (Bauer & Rosenberg 1991). In the case of thrombin inhibition, the complex formed is thrombin-antithrombin (TAT), which is rapidly removed from the circulatory compartment (t1/2 = 5 min). Complex formation occurs at a relatively slow rate in the absence of heparin. When heparin is present, however, it binds to lysyl residues on AT and dramatically accelerates the rate of complex formation (Bauer & Rosenberg 1991). The localization of a fraction of the AT bound to heparan sulfate proteoglycans (HSPG) on the endothelial surface, where enzymes of the intrinsic coagulation cascade are commonly generated, enables AT to rapidly neutralize these hemostatic enzymes and protect natural surfaces against thrombus formation (Rosenberg 1989).

Anti-inflammatory Properties of AT

Antithrombin has been shown to have significant anti-inflammatory properties. AT binds to heparin-like glucosaminoglycans on the surface of endothelial cells in vitro (Yamauchi et al 1989, Horie et al 1990) and in vivo (Harada et al 1999). This binding promotes endothelial cell release of prostacyclin (PGI2), which, in turn, impacts a variety of inflammatory processes.

It has been demonstrated that both endotoxin-induced pulmonary vascular injury (Uchiba et al 1996) and ischemia/reperfusion-induced liver injury (Harada et al 1999) were reduced by AT treatment through its promotion of endothelial PGI2 production (Figure 3). These effects were not observed with a Trp49-modified AT, which is incapable of promoting the endothelial release of PGI2 due to its lack of affinity for heparin.

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Ostrovsky et al (1997), using a cat mesentery ischemia-reperfusion injury model, showed that AT inhibits leukocyte rolling and adhesion that are characteristic of inflammatory reactions. This effect was independently confirmed by Hoffman et al (2000) and Nevière et al (2001) using intravital videomicroscopy to assess small intestine injury in endotoxemic rats treated with AT. In both cases, co-treatment with indomethacin, an inhibitor of PGI2, production, abolished the effect of AT on leukocytes. Specific interaction with cell-surface glycosaminoglycans on the endothelium is also the mechanism of antithrombin-mediated attenuation of leukocyte-endothelial cell interaction Hoffman et al (2002).

Examining the intracellular mechanism of AT anti-inflammatory activity, Oeschlager et al (2002) used cultured human monocytes and endothelial cells to show that AT inhibits NF-κB induction. Since the NF-κB transcription factor activates genes that encode defense and signaling molecules in response to various inflammatory stimuli (Hatata et al 2000), inhibition of this signaling pathway represents a plausible mechanism for AT’s anti-inflammatory effects.

**Summary of AT Properties**

In summary, it is clear that AT, in addition to its central role in the coagulation cascade, has potent anti-inflammatory activities (Figure 4). Since the anti-inflammatory properties of AT are mediated through its interaction with heparin-like receptors such as syndecan-4, located on endothelial surfaces and leukocytes, supra-physiological doses are necessary to achieve a significant effect.

**Preeclampsia**

High blood pressure is one of the most common complications in pregnancy and includes both preeclampsia and eclampsia (reviewed in Onisai et al 2009). Preeclampsia is a disorder of pregnancy developing after 20 weeks of gestation in 5-8% of pregnant women worldwide, which can cause maternal and fetal morbidity and mortality. The disorder appears to be an aberrant interaction of the placenta of the fetus with the cardiovascular and immune systems of the mother (James et al 2010). As a result, the mother develops hypertension and proteinuria and may develop disseminated intravascular coagulation (DIC), acute renal failure, stroke (ischemic, due to vasospasm and microthrombosis or even hemorrhagic due to severe thrombocytopenia), acute pulmonary edema, cerebral edema, placental abruption, liver hemorrhage/rupture, transformation into chronic hypertension, or even maternal death (preeclampsia is the second most frequent cause of maternal death linked to pregnancy). As a result of the placental insufficiency, the fetus may also be affected resulting in: pregnancy loss, fetal death *in utero*, intrauterine growth restriction (IUGR) and premature labor (Onisai et al 2009).

The causes of preeclampsia still remain somewhat elusive but are believed to be multifactorial. Preeclampsia has been described as a 2-stage disorder (reviewed in James et al 2010 and Grill et al 2009), starting with defective implantation in the 1st trimester, followed by placental-induced clinical morbidity in the 3rd trimester. Preeclampsia involves maternal gestational hypertension (systolic and diastolic blood pressure of ≥ 140 and 90 mm Hg, respectively, on two occasions, at least 6 hours...
In the asymptomatic Stage 1 of preeclampsia in the 1st trimester, the first pathophysiological occurrence is inadequate placentation characterized by incomplete decidual spiral artery remodeling (Figure 5) (reviewed in LaMarca et al. 2008; Mutter and Karumachi 2008; Onisai et al. 2009 and James et al. 2010). The spiral miometrial arteries are not correctly invaded by the fetal trophoblast and the vessels remain as small vessels, capable of vasospasm and increased vascular reactivity. Additionally, there is failure of the cytotrophoblasts to convert from a more epithelial to endothelial phenotype (Hladunewich et al. 2007). Placental ischemia occurs, followed by the release of a number of vasoactive factors (increased release of thromboxane A2 and endothelin-1 - both highly vasoconstrictive and decreased synthesis of nitric oxide (NO) and prostacyclin - both natural vasodilators). Chronic hypoxia or alternate periods of hypoxia/re-oxygenation within the intervillous space triggers tissue oxidative stress and increased placental apoptosis and necrosis.

In Stage 2, the clinical disorder arises when the maternal vascular and immune systems can no longer handle the increased shedding of placentally-produced debris (syncytiotrophoblast knots and microparticles) and the aberrant expression of pro-inflammatory, anti-angiogenic and angiogenic factors, leading to systemic endothelial cell and platelet dysfunction and an exaggerated inflammatory response (Grill et al. 2009 and James et al. 2010). In a positive feedback loop, the endothelial and platelet cells release active factors that stimulate and maintain the endothelial and platelet dysfunctions (James et al. 2010).

Biochemical and Physiological Changes Seen in Preeclampsia

In preeclampsia, a variety of biochemical and physiological changes are seen in the maternal cardiovascular and immune systems.

As reviewed in Onisai et al. (2009), the hematological changes in the cardiovascular system that occur in a preeclampsia pregnancy fall into 3 major categories:

1. The first category includes the platelet anomalies, platelet dysfunction and thrombocytopenia, which may be potentially life-threatening. The release of thromboxane A2 and decreased production of prostacyclin described above leads to an imbalance in thromboxane A2/prostacyclin ratio which favors the promotion of vasospasm, induces supplementary platelet aggregation and endothelial damage. These events contribute to the maintenance of platelet dysfunction and promote platelet consumption (activation, aggregation, microangiopathic hemolysis induced by severe vasospasm), resulting in thrombocytopenia, and an important sign of severe/aggravating preeclampsia. Excessive platelet activation is associated with endothelial dysfunction, thrombosis in microcirculation, end organ degenerative necrosis and placental infarction.

2. The second category includes alterations in hemoglobin and erythrocytic parameters due to increased endothelial permeability. In some cases, there may also be anemia due to physical destruction of erythrocytes in the microcirculation affected by disseminated microthrombosis.

3. The third category includes the coagulation changes. Preeclampsia is a highly thrombotic and procoagulant state with platelet activation and consumption, promotion of thrombin formation and promotion of fibrin formation and destruction. As compared to normal pregnant women,
Preeclamptic pregnancies show significantly increased levels of TAT complexes and PAI-1, while fibrinogen, antithrombin and PAI-2 levels are significantly reduced.

As a result of the aberrant spiral artery conversion, intermittent periods of hypoxia and reoxygenation occur in the placenta leading to oxidative stress injuries. These injuries include widespread placental lipid and protein oxidative modifications, mitochondrial and endoplasmic reticulum stress and tissue apoptosis and necrosis. The oxidative stress and endothelial activation can also stimulate IL-6 release, which increases the endothelial permeability and may reduce prostacyclin synthesis by inhibiting the cyclooxygenase. The result is an increased thromboxane A2/prostacyclin ratio, which is found in preeclampsia.

Due to the apoptosis and necrosis in the placenta, the maternal circulation is flooded with debris released from the placenta. Although such debris is usually released during the progression of a normal pregnancy, during preeclampsia, the quality and the amount of debris is much more than normal. The placental debris includes multinucleated syncytiotrophoblast knots, microparticles (STMP) and nanoparticles (exosomes). STMPs may affect both endothelial cell and lymphocyte proliferation. In vivo, STMPs bind to circulating monocytes and stimulate production of the inflammatory cytokines TNFa and IL-12 and -18, with the production of these cytokines significantly increased in STMPs from preeclamptic patients (reviewed in James et al 2010).

In addition to the hematological changes and the increased release of placental debris, a number of circulating soluble factors that contribute to endothelial dysfunction in preeclampsia have been identified (reviewed in Mutter & Karumanchi 2008 and James et al 2010). The circulating factors include cytokines (TNFa, IL-6, IL-1α, IL-1β), apoptotic factors (Fasligand, oxidized lipid products, neurokinin B and asymmetric dimethylarginine) and anti-angiogenic factors (fms-like tyrosine kinase-1 (sFlt-1) and endoglin). It has been found that the hypertension and proteinuria are due to excess circulating soluble fms-like tyrosine kinase-1 (sFlt-1), which is produced by the placenta and neutralizes vascular endothelial growth factor and placental growth factor. In addition, soluble endoglin synergizes with sFlt-1 and contributes to the pathogenesis of preeclampsia.

The inflammatory process in preeclampsia involves activation of leukocytes (possibly by STMPs), which mediate adhesion of neutrophils to the endothelium. Following activation and binding, neutrophils then transmigrate through the vascular wall releasing cytokines that mediate vasoconstriction and vascular damage, including TNFa, IL-1 and IL-8.

While a progressive increase in serum inflammatory profile is a physiological feature in a normal pregnancy, the exacerbation of this response in preeclampsia likely further contributes to the hypertensive effects of this condition, as increasing medical evidence suggests that hypertension-associated vascular disease is an inflammatory process (James et al 2010).

Treatments for Preeclampsia

In most cases of preeclampsia, safe prolongation of the pregnancy by days or weeks is highly desirable to reduce morbidity and mortality in the infant and mother. There is disagreement about management of patients with severe preeclampsia prior to 34 weeks of gestation. Antihypertensive agents, diuretics, sedatives and chronic parenteral magnesium sulfate have been used as temporizing measures in some cases (Ferrazzani 1999).

However, the only effective treatment for preeclampsia is delivery of the infant and the placenta. If part of the placenta is retained following delivery, the disorder persists. The importance of the placenta to preeclampsia is demonstrated in women with a molar pregnancy (placenta without a fetus). These women also experience preeclampsia (Mutter and Karumachi 2008).

Animal Models for Preeclampsia

Preeclampsia only occurs naturally and spontaneously in women and higher apes. It has been speculated that an upright posture and uteroplacental ischemia are necessary for development of the full spectrum of changes associated with preeclampsia in humans (Podjarny et al 2004). Although a variety of animal models have been evaluated for their relevance to the study of preeclampsia, to date, none of the models fully duplicate the events seen in the progress of preeclampsia in pregnant women (Podjarny et al 2004). In fact, in their review of the current animal models for preeclampsia, Podjarny and colleagues have concluded:
“These models are definitely of use in preeclampsia research but because this disease only occurs spontaneously in primates, the definitive studies on preeclampsia will, of necessity, be clinical.”

In spite of this, parts of the multifactorial preeclampsia syndrome have been induced in animal models, which have produced data that may be helpful in developing treatments to ameliorate this disorder in pregnant women.

**Use of Antithrombin in Animal Models for Preeclampsia or Other Relevant Disorders**

Two studies utilizing antithrombin therapy have been performed in animal models for preeclampsia (Shinyama et al 1996a, Shinyama et al 1996b). Additional animal studies for other elements involved in the preeclampsia syndrome have been conducted with AT intervention. Although these studies are not on preeclampsia per se, they may provide insight into the ability of antithrombin to ameliorate these elements of the preeclamptic cascade.


In the first model (Shinyama et al 1996a), salt-loading (2% NaCl diet) in pregnant stroke-prone spontaneously hypertensive rats caused symptoms similar to those of human preeclampsia (hypertension and proteinuria). Antithrombin (60 or 300 U/kg/d) or saline was administered intravenously for 10 days from days 9-11 to 18-20 (Figure 6). Blood pressure was measured using an automated device by the tail-cuff method on days 0, 6-8 and 15-17 of gestation. Urine was collected for 24 hours pre-conception and on days 8-10 and 17-19.

There was a significant elevation of systolic blood pressure (SBP) on days 15-17 and of urinary protein excretion on days 17-19 of gestation in salt loaded rats compared to control rats fed a normal diet.

Intravenous administration of antithrombin (60 or 300 U/kg/d) for 10 days from days 9-11 to 18-20, attenuated the aforementioned changes in blood pressure (Figure 7) and proteinuria (Figure 8) in a dose dependent manner.

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**FIGURE 6 Experimental Design (Adapted from Shinyama et al 1996a)**

- Blood pressure measurement
- Urine collection

- Blood pressure measurement

- Mating

- 0

- 6-8

- 15-17

- 18-20 (Days of gestation)

- Pre-conception

- 8-10

- 17-19

- High salt intake

- Arterial blood sampling

- Historical analysis

- Saline or AT III administration

---
AT also prevented the occurrence of arteriosclerosis and thickening of the capillary basement membrane of the kidney.

Since the changes induced in these rats by salt-loading were not due to activation of the blood coagulation system, the effect of AT in this model resulted from a mechanism largely independent of its anticoagulant action.


In the second animal model (Shinyama et al 1996b), intravascular coagulation was induced in gestation day 16-20 pregnant rats by the intravenous administration of tissue thromboplastin (TP) through the left ventricle of the heart. One hour later, organ blood flow was measured by the radioactive (¹⁸⁵Co-labeled) microsphere method and fibrin deposition in organs was measured by radiolabeling with [¹²⁵I]fibrinogen injected before the TP infusion.

TP, a lipoprotein that initiates the extrinsic coagulation pathway, caused fibrin deposition in the kidney, lung and liver, but not in the myometrium and placenta, and produced an 80% decrease in renal blood flow (RBF), with oliguria and proteinuria. TP also caused an increase in blood pressure (BP) accompanied by an increase in plasma renin activity (PRA).
Once it was established that TP increased fibrin deposition, proteinuria and blood pressure in the rats, the experiment was repeated using a prophylactic administration of AT or saline. Five minutes prior to the TP infusion in some animals, a bolus dose of AT (60 or 300 U/kg) or saline was administered intravenously followed by an AT infusion of 30 or 150 U/kg/2h, or saline, respectively.

The administration of AT in this rat model prevented the changes in fibrin deposition (Figure 10), urinary protein content (Figure 11) and blood pressure (Figure 12) in a dose-dependent manner. AT infusion also increased placental blood flow.

**FIGURE 9  Experimental Design (Adapted from Shinyama et al 1996b)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0h</td>
<td>Surgery</td>
<td>Blood pressure measurement, perfusion of heparinized saline from the left ventricle of the heart</td>
</tr>
<tr>
<td>1h</td>
<td>Urine collection (1st period)</td>
<td>Blood pressure measurement, perfusion of heparinized saline from the left ventricle of the heart</td>
</tr>
<tr>
<td>2h</td>
<td>Urine collection (2nd period)</td>
<td>Blood pressure measurement, perfusion of heparinized saline from the left ventricle of the heart</td>
</tr>
<tr>
<td></td>
<td>200 mg/kg. i.v.</td>
<td>Blood pressure measurement, perfusion of heparinized saline from the left ventricle of the heart</td>
</tr>
<tr>
<td></td>
<td>Thrombopisst in saline 9 mL/kg/hr, i.l.v.</td>
<td>Blood pressure measurement, perfusion of heparinized saline from the left ventricle of the heart</td>
</tr>
<tr>
<td></td>
<td>Co-microsphere, i.l.v.</td>
<td>Blood pressure measurement, perfusion of heparinized saline from the left ventricle of the heart</td>
</tr>
</tbody>
</table>

- I-Fibrinogen, i.v.
- Tranexamic acid 200 mg/kg, i.v.
- 9 mL/kg/hr, i.l.v.
- 0h 1h 2h
FIGURE 10  Fibrin Deposition in Kidney, Liver and Lung (Adapted from Shinya et al 1996b)

Inhibition by antithrombin III (AT III) of thromboplastin (TP)-induced enhanced fibrin deposition (top) and decreased blood flow (bottom) in the kidney, liver and lung. Fibrin deposition and blood flow in organs were measured 1h after discontinuation of TP infusion. Fibrin deposition in organs was measured with $^{125}$I fibrinogen. Blood flow was measured by the radioactive ($^{57}$Co) microsphere method. Saline intravenously (i.v.) + intra-left ventricular (i.l.v.) infusion of saline (n=7, white columns), saline i.v. + i.l.v. infusion of TP (n=7, solid orange columns).

Values are mean + SEM.

* p<0.05 and ** p<0.01 as compared with the value for saline-treated animals not receiving TP infusion (Dunnett’s test).

† p<0.05 as compared with the value for saline-treated animals receiving TP infusion (Dunnett's test).

Thromboplastin
The authors suggest that intravascular coagulation plays a significant role in the events of preeclampsia and that AT may have therapeutic potential in its treatment. Also since AT improved placental blood flow, it may help ameliorate the ischemia that is occurring in the placenta.

**Effect of AT on Prostacyclin Production, TNF-alpha and Apoptosis**

A series of *in vitro* and *in vivo* studies have been performed with antithrombin that demonstrates its ability to increase prostacyclin (PGI2) production, inhibit TNFα production and apoptosis in model systems. Prostacyclin is an important mediator of the anti-inflammatory effect of AT and is a potent inhibitor of leukocyte activation and monocyte production of TNF-alpha and the production of IL-8, two pro-inflammatory cytokines.

Mizutani et al (2003) used a rat renal ischemia/reperfusion (I/R) model to show that antithrombin inhibited leukocyte activation through promotion of an increase in prostacyclin (an inhibitor of leukocyte activation) production. A 1998 report from Okajima showed a similar effect of AT on inhibition of leukocyte activation through induction of production of prostacyclin in a rat endotoxin-induced pulmonary vascular injury model.

Harada et al (2004) used a rat hepatic ischemia/reperfusion (I/R) system to show that antithrombin induced an increase in prostacyclin production. AT treatment also increased hepatic tissue blood flow and inhibited hepatic inflammatory responses. The effects of AT were reversed by pretreatment with indomethacin a non-selective inhibitor of cyclooxygenase. The authors suggested that AT might be acting in this system by increasing the production of prostacyclin and PGE2 through activation of COX-1. Additionally, AT inhibited TNFα production. The authors concluded that these effects induced by AT may contribute to its anti-inflammatory activity.

Hirose et al (2004) demonstrated in a rat model of ischemia/reperfusion-induced spinal cord injury that AT reduces the injury by attenuating the inflammatory response. Microinfarctions of the spinal cord were markedly reduce by AT. AT treatment also increased the spinal cord tissue levels of 6-keto-PGF1α, a stable metabolite of...
endothelial cell activation

endothelial cell (HUVEC) model that AT inhibited TNFα-induced edema formation and tissue injury.

AT affects this leukocyte function in an anti-inflammatory way via heparan sulfate proteoglycans.

**Antithrombin Replacement in Human Preeclampsia**

There have been limited case reports and studies using antithrombin replacement in the setting of preeclampsia. Buller administered a dose of 2,000 Units of antithrombin (plasma) concentrate to an antithrombin deficient patient with severe preeclampsia and found that the antithrombin infusion improved blood pressure, proteinuria and coagulation parameters. The patient had an uneventful Cesarean delivery (Buller 1980).

Terao et al (1989) reported on 40 patients with preeclampsia. Twenty seven were treated with antithrombin (plasma) replacement and 13 were untreated. It is unclear how many patients experienced severe preeclampsia. Antithrombin was dosed at 1,000-2,000 units/ day. In Japan, hypertensive patients are scored on a Gestosis Index (GI), consisting of edema, proteinuria, systolic and diastolic blood pressure criteria. The authors considered that there was a 40% efficacy in the treated group vs 0% in the untreated group. They also found a correlation between GI and antithrombin activity.

Nakabayashi and colleagues evaluated antithrombin (plasma) replacement vs heparin in early onset severe preeclampsia with intrauterine fetal growth restriction under 32 weeks (Nakabayashi et al 1999). Fifteen patients received a dose of antithrombin replacement 1500IU/day x7 days. The antithrombin infusion was associated with improved systolic blood pressure and sonographic estimation of fetal weight, as compared to heparin. The authors concluded that antithrombin replacement therapy was useful for improving maternal hypertension and fetal weight in severe preeclampsia.

Kobayashi and colleagues performed a Phase II trial with 29 patients with severe preeclampsia (Gestosis index≥ 6), at 24-36wks (Kobayashi et al 2003a). The study was designed to assess the efficacy of antithrombin plasma concentrate, as a coagulation inhibitor for the treatment of severe preeclampsia. Antithrombin replacement at 1,500 units plus heparin 5000 units daily was compared to a control group who received only heparin 5000 units daily for 7 days. The Gestosis Index and biophysical profile, a fetal of fetal well being were both significantly improved with antithrombin replacement (p=0.46 and p=0.022, respectively). When the authors compared coagulation parameters, antithrombin replacement was associated with improved levels of plasmin- plasmin inhibitor.
complex, D-Dimer, and platelet counts. The authors’ impression was that the addition of antithrombin replacement was superior to heparin alone in improving maternal and fetal outcome.

Kobayashi reported on a late Phase II study which demonstrated a linear dose–response relationship for the use of antithrombin (plasma) concentrate in the treatment of severe preeclampsia. He reported improved uteroplacental circulation found with antithrombin replacement administration. The optimal dose of antithrombin replacement was 3000 units/day, which was efficacious and safe for both mother and fetus (Kobayashi 2003b, 2005).

Maki and colleagues performed a controlled double blind phase III trial, with 133 patients with severely preeclampsia, whose gestational ages ranged from 24-35wks (Maki et al 2000). The Gestosis Index was ≥ 6. Sixty six patients received antithrombin replacement at a dose of 3000 IU per day. Sixty seven patients received intravenous placebo for seven days. The patients were followed for 2 weeks until delivery. The authors found a significant prolongation of pregnancy with antithrombin replacement (16.8 +/- 2.0 v 10.2 +/- 1.2, p<0.007, and a significantly greater gestational age at delivery 34.1 +/- 3.2 vs 33.0 +/- 2.7wks). The study demonstrated a significant mean increase in gestational age of 6.5 days in the antithrombin replacement group. Both antithrombin antigen and antithrombin activity increased significantly (p value 0.001) in the antithrombin replacement group, compared to placebo. The authors concluded that antithrombin replacement in addition to conventional therapy improved maternal symptoms, improved the biophysical profile score, prolonged pregnancy and decreased prevalence of very low birth weight infants.

Paternoster performed a dosing study comparing 2 different dosing regimens of antithrombin (plasma) replacement, in the setting of severe preeclampsia between 24-33 wks (Paternoster et al 2004). The High dose antithrombin group (n=10) received 3000 IU/d x5 day (1268.000 IU total; 12600IU per pt mean dose). The Standard dose antithrombin group (n=13) received antithrombin replacement to maintain 80% antithrombin activity. They found that the Standard dose group received 43.800 IU total dose per patient, and a mean dose per patient of 3370 IU. The High dose group ultimately received 3.7x higher dose than the Standard dose group. The mean number of days the pregnancy was prolonged in the High dose group was 6 days, as compared to 3.5 days in the Standard dose group. The High dose group also had greater birthweights, (1185g vs 1005g), but this difference was not significant (p 0.3). Their conclusion was that the Standard dose of antithrombin replacement corrects the hemostatic abnormality, while the High dose also corrects inflammatory state.

In summary, there is preliminary evidence suggesting a benefit to antithrombin replacement in the setting of preeclampsia. The published studies do suggest a benefit to antithrombin replacement in preeclampsia. There is a disproportionate contribution to the literature by the Japanese studies which are confounded by a preeclamptic classification not used by the rest of the developed world. The preliminary data does not suggest harm, either for the mother or the fetus. A high quality trial is needed to address the many questions raised by these provocative preliminary studies.

Four additional clinical publications that advocate the use of AT in clinical studies in preeclampsia, are available. The first study is a PK study conducted by Weiner et al (1990) in 5 normal healthy and 5 preeclamptic pregnant women with a document deficiency of AT. Healthy pregnant women received 1500 units of antithrombin (plasma) intravenously over 20 min. Due to their low baseline AT activity of 61 to 75% of normal, preeclamptic pregnant women received 3000 units of AT intravenously. Serial blood specimens were obtained over the next 12 hr from both groups of women. The mean t1/2 of AT in the healthy group was 29.4h ±3.4h, whereas the mean t1/2 in the preeclamptic group was 8.5h ±1.2h. The significantly faster clearance in the preeclamptic group suggests rapid consumption of AT in this disorder.

The second publication by Mangione and Giarratano (2002) reports on the measurement of inflammatory cytokines (IL-6, TNF, IL-10), antithrombin, Protein C and Tissue Factor Pathway Inhibitor (TFPI) in the plasma of 36 patients with normal pregnancy and severe preeclampsia. They also measured an Organ Dysfunction Score (0-15). A correlation was found particularly between AT levels and the clinical scores. The authors indicate that they have in place in their ICU a monitoring and treatment protocol for preeclamptic patients that specifies replacement of the AT levels in the preeclamptic patient to at least 120% of normal (Figure 13). They also state that a multi-center study on AT supplementation in preeclampsia was ongoing, although to date no follow-up publication has been forthcoming with the study results.

**FIGURE 13 Protocol for AT Use in Preeclampsia** (Adapted from Mangione & Giarratano 2002)

<table>
<thead>
<tr>
<th>Protocol of monitoring and line of treatment</th>
<th>First Approach</th>
<th>General Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT III concentrates to the purpose to maintain range over 120%.</td>
<td>Fresh Frozen Plasma (from 10 to 20 ml/kg), AT III at high doses (3000-4000 IU/day), FIBRINOGEN only if under 1.25 g/l</td>
<td>Platelets if number under 70,000, No PPSB</td>
</tr>
</tbody>
</table>
A third publication by Marietta et al (2009) report on the measurement of antithrombin, platelet, fibrinogen and D-dimer levels in women with preeclampsia for several days over the time of delivery. They report that a significant drop in AT levels is associated with the clinical worsening of preeclampsia leading to the need for delivery, regardless of its severity. The AT drop was associated with a drop in platelet count and fibrinogen levels, whereas D-dimer fluctuations were not statistically significant. The AT levels in these individuals recovered immediately after parturition. AT was the only coagulation factor measured that displayed this temporal recovery after parturition. They conclude that these results strengthen the support for AT interventional trials in preeclampsia.

The last publication is a review article by Haram et al (2009) on the HELLP syndrome, which is a variant of preeclampsia. This complication of pregnancy is characterized by hemolysis, elevated liver enzyme and low platelet count (HELP) and occurs in 0.5 to 0.9% of all pregnancies and in 10–20% of cases with severe preeclampsia. The authors suggest that antithrombin (plasma) is a possible therapeutic option for treatment of preeclampsia due to its demonstrated ability to correct hypercoagulability,

**Rationale for Use of Antithrombin in Preeclampsia**

From the medical research described in the literature, it is clear that preeclampsia is a multifactorial disorder that involves both the cardiovascular and inflammatory systems. Due to its pleiotropic nature as an anticoagulant and anti-inflammatory agent, AT has been suggested as a potential treatment to ameliorate this disorder. If one considers the various features of the preeclamptic state (described and summarized in this document) and the potential impact that AT treatment might provide on these features (Table 1), it is clear that the anti-inflammatory properties of AT would most likely provide the greatest benefit in this disorder. However, one cannot underestimate the damage that thrombin and microthrombi play in placental damage and maternal cardiovascular damage.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Rationale for Use of AT in Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Observed in Preeclampsia (PE)</strong></td>
<td><strong>Potential Impact of AT Treatment</strong></td>
</tr>
<tr>
<td>Reduced levels of antithrombin thereby reducing anticoagulation; correlates with worsening of preeclampsia</td>
<td>Increase antithrombin levels to normal or supranormal levels</td>
</tr>
<tr>
<td>Elevated TAT levels</td>
<td>Should provide additional elevation in TAT levels due to hyper-coagulant state in PE</td>
</tr>
<tr>
<td>Placental infarctions due to increased thrombosis</td>
<td>Inhibits/reduces thrombosis</td>
</tr>
<tr>
<td>Fibrin deposition in maternal organs and placenta</td>
<td>Inhibits fibrin deposition</td>
</tr>
<tr>
<td>Increased thromboxane A₂ production and decreased prostacyclin production, which provides a thromboxane A₂/prostacyclin ratio which favors the promotion of vasoconstriction, induces supplementary platelet aggregation and endothelial damage</td>
<td>Increased production of prostacyclin by AT binding to heparan sulfate proteoglycans on the surface endothelial cells, which would rebalance the thromboxane A₂/prostacyclin ratio and ameliorate vasoconstriction, platelet aggregation and endothelial damage</td>
</tr>
<tr>
<td>Increased thrombin-induced vasoconstriction Increased blood pressure</td>
<td>Regulates thrombin-induced vasoconstriction Normalize blood pressure</td>
</tr>
<tr>
<td>Increased proteinuria</td>
<td>Reduces proteinuria</td>
</tr>
<tr>
<td>Increased neutrophil adhesion to the endothelium</td>
<td>Reduces neutrophil adhesion to endothelium</td>
</tr>
<tr>
<td>Release of pro-inflammatory cytokines such as TNFα and IL-6 and IL-8 that mediated vasoconstriction and vascular damage</td>
<td>Inhibits production of the pro-inflammatory cytokines</td>
</tr>
<tr>
<td>Increased IL-6 release, which increases endothelial permeability</td>
<td>Significantly inhibits production of IL-6 in inflammatory disorders</td>
</tr>
<tr>
<td>Significant platelet dysfunction</td>
<td>Reduces platelet dysfunction</td>
</tr>
<tr>
<td>Decreased placental blood flow leading to ischemia</td>
<td>Increased placental blood flow</td>
</tr>
</tbody>
</table>
CONCLUSION

A significant body of research, both non-clinical and clinical, has demonstrated the significant anticoagulant and potent anti-inflammatory properties of antithrombin. More limited non-clinical and clinical studies have shown the potential for AT therapeutic treatment of women with preeclampsia. As suggested in a number of the clinical studies cited above, the next step is the use of antithrombin in a well-controlled, randomized clinical study in women with preeclampsia.

REFERENCES


LaMarca BD, Gilbert J and Granger JP. 2008. Recent Progress Toward the Understanding of the Pathophysiology of Hypertension During Preeclampsia. Hypertension 51:982-988.


