Pathophysiology of Preeclampsia and Rationale for Heparin-mediated Extracorporeal Low-density Lipoprotein Precipitation as a Possible Therapeutic Approach

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Preface

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1. Introduction

Preeclampsia is a form of hypertension in pregnancy causing both increased maternal and fetal morbidity and mortality. It affects 2–10% nulliparas and is more prevalent when preexisting conditions such as hypertension, renal disease, or diabetes are present (August, 2000). Severe preeclampsia in pregnant women is characterized by hypertension, proteinuria, oligouria, eclampsia seizures, pulmonary edema, hepatic rupture, impaired liver function, thrombocytopenia and disseminated intravascular coagulation (Antoinette et al., 2001). Risks to the fetus include growth retardation, placental abruption and death. A major complication in some cases is characterized by hemolysis, elevated liver enzymes and low platelet count (HELLP syndrome), which is a serious, life-threatening form of preeclampsia. The incidence of HELLP syndrome has been reported to be around 0.17–0.85% of all live births (Rath, 2000), resulting in increased postpartum maternal morbidity and serious manifestations of severe preeclampsia. Till today there is no satisfactory treatment for preeclampsia except for immediate removal of the trophoblast, resulting in discontinuation of pregnancy. Prolongation of pregnancy is, in theory, favorable for the fetus but deleterious for the mother. Decisions regarding the need for treatment and the selection of specific measures should be based on assessment of relative benefits and risks for the individual mother and her fetus.

Endothelial cell activation or dysfunction appears to be mainly responsible in the pathogenesis of preeclampsia. Factors leading to endothelial dysfunction include placental ischemia, lipoprotein induced toxicity, oxygen free radicals, immune maladaptation resulting in synthesis and release of proinflammatory cytokines. Placenta, which is a heterogeneous organ, is composed of various cell types. Placental disorders, which are encountered in preeclampsia, bear similarity to the process of atherogenesis. Therefore each of these aspects will be described briefly.

1.1. Human placenta:

Human placenta is a heterogeneous organ, connecting maternal decidua with basal plate, and through umbilical vessels establishing its

contact with fetal circulation (Figure 1).
The structural and functional unit of fetal placenta is the stem villus originating at the chorionic plate and its branches becoming increasingly abundant as pregnancy advances. It brings isolated fetal bloodstream into close topographic contact with the open maternal bloodstream via coiled artery. Each stem villus with its branches has a separate fetal blood supply and this unit is termed as fetal cotyledon.

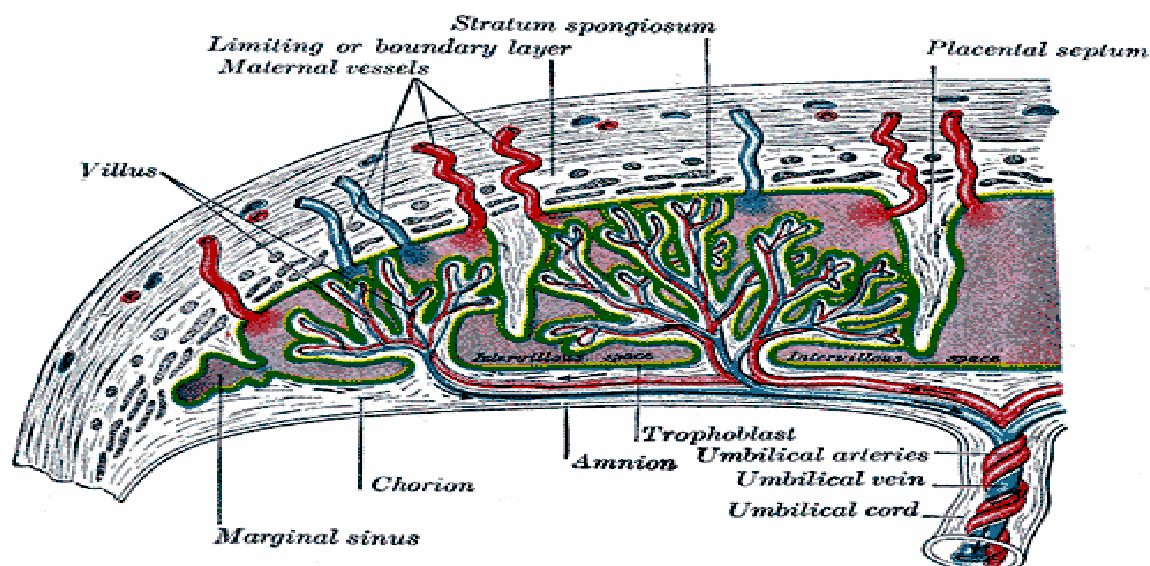


Figure 1. Schematic structure of human placenta at term (modified from Novak, 1991).

Cellular organization of multitudinous tertiary villi at term includes syncytiotrophoblast forming the syncytium on outside layer of villi, fibroblasts and Hofbauer cells together with collagen forming the stroma of the villi, and the stroma of the terminal villi include capillaries derived from umbilical arteries and veins with diameter exceeding up to 50 µm. Placental syncytium is a major regulator of transport as well as the site of synthesis of steroid, protein and peptide placental hormones. The syncytiotrophoblast is a continuous layer of multinucleated cytoplasm. Its structural-functional feature is the absence of intercellular space on its absorptive surface. All substances entering or leaving the fetal blood pass through the syncytium. In vitro studies show that syncytiotrophoblast or syncytium synthesizes and secrets estrogen, progesterone, glycoprotein hormones such as human chorionic gonadotropin (hCG), and human placental lactogen (hPL) (Kliman et al., 1986).

Hofbauer cells bear similarity to macrophages ultrastructurally and cytochemically (Castellucci et al., 1980). They posses C3, Fc receptors and HLA surface markers enabling them to participate in immune and nonimmune functions.
Placenta is the organ during pregnancy that transports numerous substrates required for the synthesis of fetal tissue and removes the waste products of fetal metabolism. The placental barrier at term is composed entirely of the fetal tissue–placental villi (Figure 2)–and varies in its thickness from 2–60 µm. Electromicroscopic studies suggest that the placental barrier can be further subdivided into five ultrastructural components: syncytium, trophoblastic basal lamina, connective tissue layer, endothelial basal lamina and endothelium. Substrate transfer between the two bloodstreams is principally regulated by the syncytial trophoblast. Small molecules are probably transferred by the microvilli along the free syncytial surface depending on their concentration gradients and at rates related to their lipid solubility and ionization whereas macromolecules are thought to taken up by pinocytosis. Membrane-limited granules in the syncytium are responsible for secretory (exocytosis) or absorptive (endocytosis) processes.

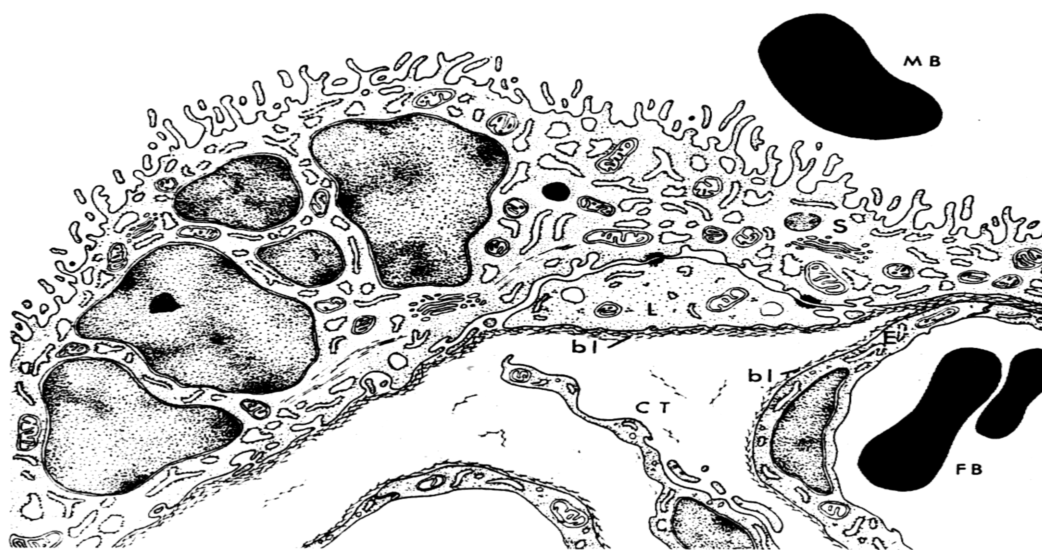


Figure 2. Human placental barrier at or approaching term. The maternal blood (MB) is separated from the fetal blood (FB) by the syncytiotrophoblast, occasional Langhans cell (L), basal lamina (bl) of the trophoblast, fetal connective tissue (CT), basal lamina (bl) of the fetal capillary and the fetal endothelium (E) (Enders, 1965).

1.2. Normal plasma lipoprotein metabolism:

Plasma lipoproteins are synthesized and secreted as micelles and serve in blood as a transport system for lipids and apolipoproteins, which are cofactors for enzymes as well as ligands for membrane receptors. The hydrophobic core of plasma lipoproteins (triglycerides and cholesterol esters) is surrounded by hydrophilic cover of cholesterol, phospholipids, and apolipoproteins.
Plasma lipoproteins are in a dynamic state and vary in their specific floating densities. They can be separated into various subclasses by ultracentrifugation. These include triglyceride-rich chylomicrons (d ≤ 0.95 g/ml) with a diameter of 80–1000 nm, triglyceride-rich very low density lipoprotein (VLDL, d ≤ 1.006 g/ml) with a diameter of 30–90 nm, cholesterol ester-rich low density lipoprotein (LDL, d = 1.019–1.063 g/ml), and protein- rich high density lipoprotein (HDL, d=1.063–1.21 g/ml) with a diameter of 8–12 nm (Havel, 1975).
Depending on their electrophoretic mobility, they are also classified as α (HDL), pre-β (VLDL) and β (LDL) lipoproteins. Chylomicrons do not show any electrophoretic mobility (Wieland and Seidel, 1977).
The main organ for synthesis of apolipoproteins is liver (apo A, apo B-100, apo-C, apo-E and apo (a)) and intestinal mucosa (apo A and apo B-48) [Scanu, 1979; Smith et al., 1978]. These apolipoproteins can be transferred from one lipoprotein class to another by enzymatic reactions. The most important function of apolipoproteins is their function as ligands for cell specific receptors and therefore determine the metabolic pathways of lipoproteins (Mahley et al., 1984).

Chylomicrons: They are synthesized by enterocytes, secreted into lymphatic circulation and via thoracic duct into blood circulation. They transport about 90% of dietary triglycerides and contain about 2–3% of cholesterol. The main apolipoproteins of chylomicrons are apo B-48, A-I, A-II, and A-IV. Due to transfer mechanism from HDL, they are also enriched with apo C and apo E. Chylomicrons are hydrolyzed to chylomicron remnants, which are avidly taken up by hepatocytes via high affinity binding receptors for apo E. These remnant particles after their hepatic uptake are hydrolyzed in lysosomes and cholesterol released suppresses both the intracellular

cholesterol synthesis and hepatic LDL receptor activity leading to indirect increase in plasma LDL levels (Zilversmit, 1979).

VLDL: The main source of supply of triglycerides for energy demands, phospholipids for membrane formation and eicosanoid synthesis as well as cholesterol for cell membrane formation and steroid hormone synthesis occurs through endogenous metabolic cascade which starts witht the formation of VLDL by the liver. The essential apolipoprotein of this lipoprotein is apo B-100 which is synthesized exclusively by the liver with the exception of fetal enterocytes.

VLDL secreted by liver is triglyceride-rich (50% particle mass) and contains relatively less cholesterol. After lipolysis by lipoprotein lipase, VLDL remnants are taken up slowly by hepatic receptors due to their apo B-100. In contrast apo E-rich VLDL particles are taken up very avidly. These remnant particles are also termed as intermediate density lipoproteins (IDL, d=1.006–1.019 g/ml). VLDL remnants, which escape uptake by liver, are hydrolyzed by hepatic lipase present in sinusoidal cells to cholesterol-rich low density lipoprotein (LDL).

LDL: The main apolipoprotein component of LDL is apo B-100 and these particles contain about 45% cholesterol and carry about 80% of circulating cholesterol. In contrast to apo E- rich lipoproteins, LDL is taken up with relatively low affinity by hepatic and extrahepatic LDL receptors (Goldstein and Brown, 1977). This explains their longer stay in circulation (2.5–3 days). LDL receptor directly regulates cholesterol uptake.

Human LDL receptor is a membrane-bound glycoprotein composed of 839 amino acids (Schneider et al., 1982; Cummings et al., 1983). The function of LDL receptor is determined by five domains i.e. ligand binding domain, a domain with 35% homology to epidermal growth factor (EGF), sugar domain with bound oxygen, a transmembrane domain and cytoplasmic domain (Innerarity et al., 1991; Brown and Goldstein, 1986).

LDL receptor binds apo B-100, a 514 kDa glycoprotein and apo E, a 34 kDa protein which is present in many copies in VLDL and certain HDL subclass.

Most LDL receptors are located in hepatic tissue and relatively few

receptors are found in extrahepatic tissue. Hepatic LDL receptors are distributed uniformly over sinusoidal surface of hepatocytes particularly in microvilli and intermicrovillous membrane (Pathak et al., 1990). In contrast, LDL receptors of extrahepatic tissues are located in coated pits. LDL is bound by coated pits, internalized and localized with endosomes. LDL is ultimately hydrolyzed in lysosomes and cholesterol released can be utilized for membrane formation, synthesis of steroid hormone and bile acid synthesis. Increased intracellular cholesterol due to LDL uptake via LDL receptor inhibits cholesterol synthesis and LDL receptor formation and activity. Thus intracellular cholesterol homeostasis is regulated by a cellular feedback mechanism. Excess of intracellular cholesterol inhibits 3-hydroxy-3-methylglutaryl CoA reductase (HMG CoA- reductase), a key enzyme for cholesterol synthesis, activates acetyl CoA-cholesterol acyl transferase (ACAT) which esterifies cholesterol leading to cholesterol esters deposition and depression of the transcription of mRNA for LDL receptor (Goldstein et al., 1974; Brown and Goldstein, 1975).

HDL: HDL is not directly secreted but is formed in plasma as opposed to nascent HDL. Apo A-I and A-II are its main protein components. Light and heavy HDL can be separated by ultracentrifugation at hydrated density of 1.063–1.12 g/ml (HDL_2) and 1.12–1.21 g/ml (HDL_3) respectively. HDL_2 is richer in cholesterol and phospholipids and contains lesser apolipoproteins than HDL_3 (Eisenberg, 1984).

Apo A-I is synthesized in intestine and liver whereas apo A-II is synthesized exclusively in liver. The discoidal phospholipid apoprotein A-I complexs are also called nascent HDL. Other sources of these particles are chylomicron and VLDL degradation products. Acquired cholesterol from cell membranes or other lipoproteins serves as substrate for lecithin:cholesterol acyltransferase (LCAT), which is found exclusively in plasma (Fielding et al., 1982). LCAT esterifies free cholesterol to cholesterol esters in the presence of apo A-I as a cofactor.

Cholesterol ester transfer protein (CETP) facilitates transfer of cholesterol esters and triglycerides from the core of HDL to VLDL, IDL or LDL. The main transfer of excess cholesterol from membranes

of periphery is mediated by CETP from HDL to IDL and LDL. Cholesterol so transferred from tissues, is ultimately taken up by the liver via LDL receptor (Havel and Hamilton, 1990).

Lipoprotein (a) [Lp(a)]: Since its discovery by Berg in 1963, detailed studies suggest that increased plasma levels of lipoprotein(a) [Lp(a)] is associated with an increased risk for cardiovascular disease (Scanu and Fless, 1990). Lp(a) resembles LDL, the major carrier of plasma cholesterol, and contains 1 mol of apo B-100 protein per particle. It is distinguished from LDL by 1 or 2 mol of apoprotein(a) that are associated through disulfide linkage with apo B-100 protein. Apoprotein(a) demonstrated striking sequence homology to plasminogen (Mclean et al., 1987) and mediates interaction of Lp(a) with plasminogen cell surface sites on endothelial cells and monocytes (Miles et al., 1989; Hajjar et al., 1989). These interactions of Lp(a) might explain the role of Lp(a) as a prothrombotic factor which interferes with the physiological functions of plasminogen. Mechanisms for uptake of Lp(a) remain still exclusive because LDL receptor does not play a major role in the uptake of Lp(a) [Armstrong et al., 1985, 1990]. This is further strengthened by the finding that Lp(a) levels remain fairly constant in humans during LDL lowering drug therapies.
Plasma levels of Lp(a) vary considerably in human due to autosomal dominant inheritance pattern (Berg and Mohr, 1963; Heiberg and Berg, 1974). Lp(a) levels increase in various diseases including nephrotic syndrome and preeclampsia probably as an acute phase response (Grone et al., 1990; Thiery et al., 1996; Husby et al., 1996). The possible implications of Lp(a) in coronary and cerebrovascular atherosclerosis can be summarized as follows:
a). Lp(a) may contribute to the development of foam cells in atherosclerotic lesions by binding to macrophages through scavenger receptor (Zioncheck et al., 1991; Haberland et al., 1992).
b). At high concentrations, Lp(a) may interfere with the fibrinolytic process by competing with plasminogen binding to the surface of endothelial cells, platelets and monocytes (Miles et al., 1989; Hajjar et al., 1989; Ezratty et al., 1993).
c). Lp(a) has been shown by in vitro studies to compete with the activation of plasminogen (Edelberg et al., 1990), and with

plasminogen for binding to fibrinogen or fibrin (Harper and Saunders, 1981; Rouy et al., 1992).

Modified lipoproteins and foam cell formation: Cholesterol deposited in foam cells (monocytes, macrophages or arterial smooth muscle cells) is derived from circulating cholesterol (Brown et al., 1980). However monocytes, macrophages and arterial smooth muscle cells do not accumulate cholesterol upon incubation with high concentrations of native LDL (Goldstein et al., 1979; Kodama et al., 1988). Chemically modified LDL by acetylation, treatment with acetic anhydride and malonaldehyde is taken up by macrophages avidly and leads to cholesterol deposition and foam cell formation. These modified LDLs are not taken up by LDL receptor but another receptor named as scavenger receptor (Kodma et al., 1988). Macrophages and endothelial cells and possibly placental cells have potential to oxidatively modify native LDL (Steinbrecher et al., 1989; Bonet et al., 1995, 1998). Selective retention of LDL in arterial wall probably by adhering to arterial proteoglycan enhances its susceptibility to oxidative modification (Hurt-Camejo et al., 1992). In addition to scavenger receptor another receptor CD36 is also important in the uptake of modified lipoproteins (Endemann et al., 1993). Modified LDL brings about profound alteration in expression of factors, which play important roles in atherogenesis and vascular diseases (Table 1).
Lp(a) is also readily oxidized and oxidized Lp(a) has been reported to impair endothelial function more strongly than LDL (Galle et al., 1998).

Table 1. Functions of modified LDL

Functions:
Induces monocyte binding to endothelial cells
Increases tissue factor activity, expression of monocyte colony stimulating factor, monocyte chemoattractant protein-1, vascular cellular adhesion molecule-1
Induces apoptosis
Induces expression of interleukins 1 and 8
Inhibits nitric oxide release
Increases collagen synthesis in smooth muscle cells
Increases intracellular calcium
Activates nuclear factor – kappa B
Induces expression of type 1 metalloproteinase

(adapted from Steinberg, 2002).

1.3. Alterations in lipoproteins, coagulatory factors and proinflammatory markers in preeclampsia:

Lipoproteins: In early nineteen century, Becquerel and Rodier (1844) and Virchow (1847) observed elevation of plasma lipids in pregnancy. Plasma total cholesterol, LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), triglycerides and nonesterfied fatty acid (FFA) levels were reported to be increased to varying degree (Metzger, 1982). A progressive increase in VLDL-cholesterol (VLDL-C) and LDL-C has also been reported during the whole course of pregnancy (Desoye et al., 1987; Steitz et al., 1987). However HDL-C reaches a peak in mid-gestation (20th gestational week) and then declines in late gestation (Fahraeus et al., 1985). Changes in plasma Lp(a) follow a similar pattern (Zechner et al., 1986).

Several mechanisms specific for pregnancy seem to be responsible for these phenomena. First, elevated estrogen levels during gestation and insulin resistance at late gestation increases fat mobilization from adipose tissue resulting in an increased hepatic synthesis of triglyceride-rich VLDL from the liver. Secondly, both LPL and hepatic lipase activity are reduced in pregnancy resulting in elevated circualting levels of triglycerides, VLDL, and IDL, and also enrichment of LDL and HDL with triglycerides due to activated CETP

particularly at late gestation (Satter et al., 1997; Hubel et al., 1998).
Preeclampsia is associated with hypertriglyceridemia (Potter and Nestel, 1979) and more recent studies report that triglycerides and free fatty acids are already elevated in the first and second trimester in these women (Lorentzen et al., 1994; Gratacos et al., 1996; Endresen et al., 1994). Murai et al. (1997) reported that body mass index, circulating triglycerides and nonesterified fatty acid levels were significantly increased in preeclamptic patients compared to normal pregnant women. Thadhani et al. (1998) reported an increased relative risk of preeclampsia in women with hypercholesterolemia as compared to those with only gestational hypertension. Several studies reported high plasma levels of atherogenic small dense LDL in women with preeclampsia (Hubel et al., 1998; Sattar et al., 1997) which are susceptible to oxidative modification and exert deleterious effects on endothelium. These smaller and denser LDL particles are less efficiently internalized by LDL receptor. Husby et al. (1996) reported a family with two severe preeclampsia /eclampsia patients with high levels of lipoprotein (a), and this finding was confirmed in a larger cohort by others (Kaminski et al., 2000). High levels of Lp(a) might lead to deposition of fibrin in the uterine spiral arteries in pregnancy.
Preeclampsia and atherosclerosis share common important risk factors such as hypertension, obesity, diabetes, and hyperhomocysteinemia (Pipkin, 1999). The term “acute atherosis” was introduced in classic studies describing vascular lesions in the placental bed of women with preeclampsia because of the presence of lipid-laden macrophages, as observed in the atherosclerotic plaque (DeWolf et al., 1975). Preeclampsia could thus represent an acute model of endothelial cell activation and subsequent interaction of lipid peroxides in combination with other endothelial and proinflammatory stimuli.
Fetus is constantly infused with substrates from the placenta, however after birth neonate utilizes endogenous substrates for glucose homeostasis. In order to meet these demands, fetus stores glycogen and lipids and transfer of lipids from maternal circulation via placenta is essential.

Coagulation during preeclamsia: In addition to circulating fibrinogen levels, other clotting factors including prothrombin and factors V, VII, VIII, IX, X and XII are elevated during pregnancy

(Table 2) [Bonnar and Hathaway, 1981]. Despite increased risk of thrombin formation, compensatory rise in anti-thrombin III is inadequate in pregnancy (Howie et al., 1971).

Table 2. Comparison of coagulatory factors and inhibitors between non-pregnancy and late pregnancy

Factor	Non-pregnancy	Late pregnancy
Fibrinogen	2- 4.5g/L	4.0- 6.5g/L
Factor II	75- 125%	100- 125%
Factor V	75- 125%	100- 150%
Factor VII	75- 125%	150- 250%
Factor VIII	75- 150%	200- 500%
Factor IX	75- 125%	100- 150%
Factor X	75- 125%	150- 200%
Factor XI	75- 125%	50- 100%
Factor XII	75- 125%	100- 200%
Factor XIII	75- 125%	35- 75%
Antithrombin III	85- 110%	75- 100%
Antifactor Xa	85- 110%	75- 100%

The platelet count shows little change during the course of normal pregnancy (Shaper et al., 1968; Rakoczi et al., 1979).
Plasma plasminogen levels are also increased during pregnancy. Together with elevated levels of fibrinogen, potential for generation of plasmin is therefore present in pregnancy (Bonnar et al., 1969). However compensatory rise of antiplasmin may balance the plasmin formation.
Coagulatory and fibrinolytic maladjustment plays an important role in the pathogenesis of preclampsia. Hypercoagulability may be associated with features such as fibrin deposition in various organs, consumptive thrombocytopenia, and placental hypoperfusion, insufficiency and infarction observed in placentas from preeclamptic patients (Schjetlein et al., 1997; Graeff et al., 1984). Fibrin deposition is typically observed in the uteroplacental blood stream. High levels of fibrin and fibrin(ogen) degradative products and fibrin oligomers, in combination with thrombocytopenia and factor VIII consumption have been interpreted as an additional evidence for the significance of intravascular clotting in the pathogenesis of gestosis (Kobayashi et al.,

1999). Hemolysis is the consequence of impairment in microcirculation. Thrombocytopenia which precedes the elevation of circulatory fibrin(ogen) degradation products, also suggests the disturbed interrelation between platelets and endothelium.

NF-kappa B: Nuclear factor kappa-B (NF-κB) is a central transcription factor involved in inflammatory processes and its activation leads to enhanced expression of various proinflammatory biochemical markers including cytokines, chemokines and adhesion molecules (Baeuerle and Henkel, 1994; Baldwin, 1996). The first step in this cascade is activation of cytoplasmic inactive dimers of NF-κB which are bound to I-kappa B. I-kappa B is phosphorylated, ubiquitinated and ultimately degraded by proteasomes. Unbound dimers so formed are translocated into nucleus, bind to DNA and activate inflammatory genes. The central role of NF-κB activation is underlined by the findings that specific inhibition of the activation of this transcription factor by steroids not only inhibits TNF-release into circulation but also reduces mortality in animal models treated with lethal doses of endotoxin (Siebenlist et al., 1994). Also an activation of NF-κB was noted in PBMC from patients with sepsis and it correlated with mortality (Bohrer et al., 1997). At a molecular level NF-κB is composed of two monomers of Rel family which include classical Rel A (p65), C-Rel, RelB, p50, and p52. Even though most of these dimers have been described, p65/p50 heterodimer is the main heterodimer responsible for signal transduction in humans (Ghosh, 1999). The binding of this dimer to DNA leads to expression of proinflammatory cytokines including IL-1, TNF, growth factors (PDGF, TGF), colony stimulating factors (CSF), adhesion molecules (ICAM, VCAM), vasoactive mediators (iNOS, NO), complement factors, cytokine receptors and acute phase proteins (Christman et al., 1998).

AP-1: Activator protein-1 (AP-1) is a heterodimer of Fos and Jun oncoproteins belonging to leucine zipper (bZIP) family and is another important transcription factor. Even though various transcription factors of Fos (c-Fos, FosB, Fra-1, and Fra-2) and Jun (c-Jun, JunB and JunD) family bound by leucine zipper have been described, Fos/Jun heterodimer is the major form of AP-1 (Abate and Curran, 1990). AP-1, like NF-κB, activates number of genes encoding

inflammatory proteins. Some genes which require simultaneous activation of NF-κB already show a basal activation with inflammatory stimulation. The possible reason for this situation may be due to involvement of AP-1 in cell homeostasis, cell growth and cell differentiation (Lian et al., 1991; Karin et al., 1997).

Underlying mechanisms in preeclampsia which is a life-threatening condition for both mother and fetus are alterations in maternal hemodynamics, endothelial function, and maternal inflammatory response (Granger et al., 2001; VanWijk et al., 2000; Roberts et al., 2002). Elevated biochemical markers of endothelial and inflammatory activation have already been reported during the first and early second trimesters in women who later develop preeclampsia. Most of studies so far reported increased circulating levels of TNF, soluble TNF receptor p75, and p55, IL-6, and IL-8 (Meekins, 1995; Visser et al., 2002; Kauma et al., 2002). Furthermore increased serum levels of adhesion molecules including E-selectin, VCAM-1 and ICAM-1 have been noted in preeclamptic women (Austgulen et al., 1997). Increased circulating levels of these molecules reflects their increased expression on endothelial cells and may be responsible for leukocyte activation in these patients. Newborns of preeclamptic mothers have increased expression of CD15s, CD49d/CD29 and CD31 on neutrophils and CD15s, CD11c and CD54 on monocytes (Mellembakken et al., 2001). This activation of neutrophils and monocytes during preeclampsia involving enhanced chemokine activation probably have deleterious effects on fetus.

1.4. Summary of existing information and purpose of the present study:

Chronic hypertension, diabetes and obesity are predisposing factors for preeclampsia. It is characterized by hypertension, proteinuria, oligouria, eclampsia seizures, impaired liver function, thrombocytopenia and coagulatory disorders. The link between obesity and preeclampsia is unclear. Obesity is characterized by expanded blood volume, increased cardiac output and hypertension. Elevated cardiac output with compensatory vasodilation may expose capillary beds to systemic pressure and increased blood flow

eventually leading to endothelial dysfunction which plays a central role in preeclampsia. Alterations in lipoprotein profile and concentrations particularly LDL, Lp(a) and VLDL have been associated with this condition, which is life-threatening for both mother and fetus.
No satisfactory treatment for preeclampsia except immediate removal of trophoblast resulting in discontinuation of pregnancy is available till date. Drug therapies, including antihypertensive, anti-eclampsia, vasomodulators, antithrombotic and anti- coagulatory have not be very promising. A therapeutic approach which has potential to reduce the circulating concentrations of LDL, Lp(a), fibrinogen, proinflammatory markers, procoagu- latory and prothrombotic factors deserves attention.
Heparin-mediated extracorporeal low-density lipoprotein precipitation (H.E.L.P.) therapy drastically reduces LDL, Lp(a) and fibrinogen. Furthermore it improves procoagulatory and prothrombotic situation in coronary heart disease (CHD) patients. Since atheroslcerosis and preeclampsia share certain predisposing factors, the present study was undertaken to examine the potential of H.E.L.P.-apheresis as a possible therapy in patients with preeclampsia.

Following questions were addressed in this investigation:
a). Does lipoprotein profile, pattern and lipid-protein composition of plasma lipoproteins isolated by sequential ultracentrifugation and circulating proinflmamatory cytokines show differences in normal pregnant women undergoing cesarean section and preeclamptic patients?
b). Is H.E.L.P. therapy which effectively reduces LDL, Lp(a) and fibrinogen and improves the coagulatory situation and plasma viscosity in CHD patietns also tolerable and without any adverse events in preeclamptic patients?
c). Does the reduction of circulating plasma lipoproteins, pro-inflammatory markers and improvement of coagulatory and thrombotic situation result in positive outcome for the preeclamptic mother and neonate?

This investigation may be useful in designing therapeutic concepts for patients with preeclampsia.

Zielsetzung

Hypertonie, Diabetes und Adipositas stellen prädisponierende Faktoren der Präeklampsie dar. Klinische und klinisch-chemische Charakteristika der Präeklampsie sind Protein- und Oligurie, Krampfanfälle, Leberfunktionsstörungen, Thrombozytopenie sowie Gerinnungsstörungen. Der Zusammenhang zwischen Übergewicht und Präeklampsie ist bisher unklar. Übergewicht ist jedoch mit einem erhöhten Blutvolumen, einem erhöhten Herzzeitvolumen und Bluthochdruck assoziiert. Das erhöhte Herzzeitvolumen könnte, in Verbindung mit der kompensatorischen Vasodilatation, über eine Druckerhöhung im Kapillarbett und einem gesteigerten Kapillarfluß, zu einer Endotheldysfunktion führen die eine wesentliche Rolle bei der Entstehung der Präeklampsie spielt. Die Präeklampsie, welche eine lebensbedrohliche Komplikation für Mutter und Kind in der Schwangerschaft darstellt, wurde außerdem mit Veränderungen im Lipoproteinprofil - insbesondere Erhöhung von LDL, VLDL und Lp(a) - in Verbindung gebracht.

Außer einem sofortigen Schwangerschaftsabbruch gibt es bis heute kein adäquates therapeutisches Konzept zur Behandlung einer Präeklampsie. Antihypertensiva, insbesondere Modulatoren des Gefäßtonus, antithrombotische und antikoagulatorische Medikamente haben sich als nicht hilfreich erwiesen. Aus diesem Grund scheint der Versuch einer therapeutischen Senkung von LDL, Lp(a) und Fibrinogen in Verbindung mit proinflammatorischen, procoagulatorischen und prothrombotischen Mediatoren vielversprechend. Die Heparininduzierte Extrakorporale LDL-Präzipitation (H.E.L.P.) führt zu einer drastischen Reduktion von LDL, Lp(a) und Fibrinogen. Darüber hinaus führt die H.E.L.P.-Therapie bei KHK-Patienten zu einer Verbesserung der procoagulatorischen und prothrombotischen Situation. Da die Atherosklerose und die Präeklampsie somit gemeinsame prädisponierende Faktoren aufweisen, wurde in der vorliegenden Studie die Wirksamkeit der H.E.L.P.-Apherese als therapeutische Option in der Behandlung der Präeklampsie untersucht.

Folgende Fragen sollten mit dieser Studie beantwortet werden:

1. Unterscheiden sich Lipoproteinprofil, -Konzentration und Zusammensetzung der isolierten Lipoproteine sowie die Konzentration proinflammatorischer Marker bei Patientinnen mit Präeklampsie von denen schwangerer Frauen die durch eine Sektio entbunden werden.
2. Wird die H.E.L.P.-Therapie, mit Hilfe derer man sehr effektiv die Plasmaspiegel von LDL, Lp(a) und Fibrinogen absenken kann, von den schwer kranken Patientinnen ohne Nebenwirkungen toleriert.
3. Führt die durch die H.E.L.P.-Apheresetherapie induzierte Reduktion von Plasmalipoproteinen und proinflammatorischen Mediatoren sowie die Verbesserung des prokoagulatorischen und prothrombotischen Zustands auch zu einer Verbesserung des Outcomes für Mutter und Kind.

Diese Untersuchungen könnten als Basis zur Planung und Durchführung von Studien zur Behandlung der Präeklampsie dienen.

2. Methods

2.1. Subjects:

CHD Patients: In order to establish whether H.E.L.P. therapy reduces the levels of proinflammatory cytokines, adhesion molecules and improves coagulatory situation, we measured these parameters in coronary heart disease (CHD) patients undergoing regular H.E.L.P.-apheresis immediately before and after each therapy. Twenty-two patients (18 men and 4 women, mean age 57.3 ± 10.9 years) suffering from CHD were included in this pilot study. Eleven of these patients were on Statins and four had undergone heart transplantation 3 to 6 years ago.

Preeclamptic and normal pregnant subjects: A controlled study was designed to evaluate lipid and proinflammatory situation in both normal and preeclamptic patients as well as in newborns. The ethics committee of University Munich approved this study and all the patients enrolled gave their written consent to participate in this study. Preeclamptic patients (n=6, age 32.4 ± 5.8 years, gestational age 27.1 ± 3.1 weeks) were selected strictly according to guidelines of American College of Obstetricians and Gynecologists (ACOG) (1996) i,e onset of hypertension during pregnancy (BP≥ 140/90 mmHg on two occasions ≥ 6 h apart after 20 weeks' gestation) and detectable urinary protein (≥ 1+ by dipstick or ≥ 300 mg/24h). Women with preexisting chronic hypertension (BP greater than 140/90 mmHg), or requiring antihypertensive medications before pregnancy or before 20 weeks of gestation were excluded. Furthermore, women with preexisting chronic renal disease, diabetes or pregnancy-related diabetes and inflammatory conditions, such as connective tissue disease or cystic fibrosis, were not included in this study. Three of the 6 preeclamptic patients were treated by H.E.L.P. therapy.
Pregnant women without any clinical symptoms (n=44, age 32.4 ± 5.8 years, gestational age 37.6 ± 1.9 weeks), but requiring cesarean delivery for conditions such as breech presentation or repeated elective cesarean delivery were also included in this study and served as controls. These patients had no proteinuria, hypertension and other complications during pregnancy and were not on any medication.

2.2. Sampling:

2.2.1. Blood collection:

Blood from CHD patients was collected immediately before and after single H.E.L.P.-apheresis to obtain serum, citrate and EDTA plasma. Blood samples were centrifuged within 1 hour at 1000 g, 4°C, for 15 minutes. All the serum and plasma samples were aliquoted and stored at -80°C for later analysis.
Maternal blood was collected to obtain serum, citrate and EDTA plasma immediately after anesthesia and before the cesarean sections. Placental blood was collected under sterile conditions from the placental venous vessels immediately after the separation of placenta from maternal uterus. For isolation of peripheral blood mononuclear cells (PBMC), EDTA blood was kept at room temperature and cells were isolated within 1 hour. Other blood samples were centrifuged immediately at 4°C for 15 minutes. Serum and plasma were aliquoted and stored at
-80°C until use. Routine clinical chemical, lipid and lipoprotein analysis, as well as lipoprotein isolation by sequential ultracentrifugation were performed on the same day.

2.2.2. Placental tissue:

Immediately after separation from maternal uterus, placenta was placed on a sterilized polyacrylamide platform and extended to its natural size. Full thickness punch biopsy was taken approximately 3 cm distant from the umbilical cord without any visible ischemic changes, and was frozen immediately in liquid nitrogen (-180°C) for immunohistochemical and biochemical studies.

2.3. Mononuclear cells:

Cells were isolated from EDTA blood (10–15 ml) using VACUTAINER® tube (Becton Dickinson, USA) and centrifuged at 1500 g for 20 minutes. PBMC containing interface was collected, resuspended in Phosphate buffered saline (PBS) and recentrifuged

(400 g, for 10 minutes) to wash the cells. Cells were then recentrifuged and pellets were stored at -80°C until use.

2.4. Serum lipids and lipoproteins:

Serum lipid profile (total cholesterol, phospholipids, triglycerides, LDL-C, HDL-C, VLDL-C, Lp(a), apo AI and apoB) were measured by routine Automatic Analyzer (Hitachi 911, Roche) methods used at the Institute of Clinical Chemistry, Klinikum Grosshadern, München. Nonesterified fatty acids (FFA) were measured by NEFA C kit (Wako, Germany). Fibrinogen was measured according to Clauss method (STA-R, Roche).

2.5. Lipoprotein isolation:

EDTA plasma was brought to density of 1.019 g/ml by addition of solid NaCl and centrifuged at 150, 000 g at 4°C for 24 hours. Floating lipoproteins (VLDL and IDL) were collected by tube slicing technique. Infranate was then brought to the density of 1.063 g/ml with NaCl to float LDL (180,000 g, 4°C for 24 hours). After collecting the floating LDL, infranate was brought to density of 1.21 g/ml by addition of solid KBr and centrifuged at 200,000 g (48 hours at 4°C) to float HDL (Havel et al., 1955). All lipoproteins fractions were recentrifuged at their respective floating densities to concentrate them. Lipoproteins fractions so obtained were dialyzed against 5mM Tris buffer pH 7.4, containing 154 mM NaCl and 0.25 mM EDTA. Lipoprotein fractions were analyzed for their lipid-protein composition, apolipoprotein profile by electrophoresis on polyacrylamide gel in the presence of sodium dodecyl sulfate (SDS-Page) [Walli, 1984].

2.6. H.E.L.P. therapy procedure:

The procedure for H.E.L.P. therapy has been described previously (Eisenhauer et al., 1987; Seidel, 1994). In brief, venous blood was drawn from cubital vein catheter and blood cells were separated from plasma which was then mixed with acetate-heparin buffer (pH 4.85 and 1:1 ratio) to precipitate fibrinogen, LDL and Lp(a). This

suspension was continuously recirculated to remove precipitated fibrinogen, LDL and Lp(a). Thereafter excess of heparin was removed by anion-exchange filter. Finally physiological pH was restored by bicarbonate dialysis and extra fluid was removed by ultrafiltration. In H.E.L.P. Secura system, dialysis solution was prepared by dialysis fluid module which requires reverse osmosis device and external water supply. Plasma free from LDL, fibrinogen and Lp(a) was mixed with cell-rich blood fraction and returned back to the patients. Recently developed Plasmat Futura system is based on the same principle as Secura but recirculation circuit of precipitated lipoproteins and fibrinogen suspension is omitted. Furthermore, reverse osmosis device has been replaced by ready to use sterile dialysis solution (Blessing et al., 2004). The average plasma volume treated by both systems was 3300 ml per session.

2.7. Immunohistochemical techniques:

Tissue samples, 4-6 µm in thickness, were prepared using cryostat technology (CM1900, Leica) and mounted on poly-L-lysine coated slides. Slides were dried at room temperature for 1 hour, fixed in acetone for 5 minutes, air-dried and kept at -20°C until use. Stainings for each antibody were performed on the same day.

Immunofluorescence: Placental tissue sections were rehydrated in humid chamber for 20 minutes. Both primary and secondary antibodies were diluted to 1:200 and 1:50 respectively with PBS containing 1% BSA (pH 7.2). Negative controls for each staining were run in the absence of primary antibody.

Cytokeratin. Tissue section was covered with primary antibody mouse-antihuman cytokeratin (DAKO, Germany) and incubated at room temperature for 1 hour in humid chamber. Thereafter slides were washed 2 times for 10 minutes each with PBS containing 0.2% Tween 20 before incubating them with secondary antibody anti-mouse IgG FITC conjungate (Sigma). Slides was then air-dried, mounted with Glycerin medium pH 8.4 (Euroimmun), covered by a cover glass and sealed with transparent nail lacquer. Slides were examined under Leitz DMRXE-microscope (Leica, Wetzler) with selective filters to observe the counterstaining with DAPI (4’,6-Diamidino-2- Phenylindole).

Ektachrom-Film 400 ASA (Kodak, Stuttgart) was used for photographs.

Von Willebrand factor (factor VIII) and Desmin. Primary antibody rabbit-antihuman von Willebrand factor (DAKO) was used. Secondary antibody, anti-rabbit IgG FITC conjugate (Sigma), was diluted 1:50. Primary antibody for desmin staining was mouse anti-human (Boehringer, Manheim) diluted 1:200 and secondary antibody used was anti-mouse IgG FITC conjugate (Sigma). Rest of procedures for both the factors were as described above.

NF-kappa B. Primary antibody used was monoclonal mouse NF-κB P65 subunit (Mab 3026, Chemicon, Temecula, CA). After 1 hour incubation with primary antibody, secondary antibody, anti-mouse IgG FITC conjugate (Sigma), was applied for 30 minutes. Thereafter slides were washed and counterstained with DAPI (5μg/ml) for 10 minutes, washed, mounted and visualized.

Immunocytochemistry: Frozen tissue sections were rehydrated in humid chamber for 20 minutes, immersed in Sodium citrate buffer (10 mM, pH 6.0), incubated in a microwave oven (700 watt) twice for 5 minutes each to inactivate endogenous peroxidase. Slides were sequentially washed under running tap water for 30 minutes then immersed twice in PBS containing Tween for 10 minutes each. They were marked with DAKO pen and unspecific binding was blocked with goat serum for 10 minutes at room temperature. Primary antibody mouse anti-human eNOS IgG1 (Transduction laboratories, Lexington, KY) was diluted 1:200 with PBS containing 0.05% Tween and 1% BSA. They were incubated at room temperature for 2 hours followed by washing in PBS containing 0.05% Tween for 10 minutes twice. Biotinylated rabbit anti-mouse immunoglobulins (E0354, DAKO), diluted 1:200 in PBS-BSA served as a secondary antibody. After 30 minutes incubation, slides were washed twice in PBS-Tween for 10 minutes each. This was followed by incubation with streptavidin-horse reddish peroxidase (HRP) (P0397, DAKO) diluted 1:200 for 20 minutes. Substrate 3-amino-9-ethyl -carbazole (ACE) (0.01%) containing 0.006% H_2O_2 added shortly before use was used for color development. Sections were counterstained with Harris modified Hematoxylin 1:4 (Sigma) for 30 seconds. Slides were examined under light microscope and film EPY 64T (Kodak) was used

for photographs.

2.8. Transcription factors:

Nuclear extracts from PBMC and placental tissue were prepared by modification of previously described methods [Schrieber et al., 1989; Brand et al., 1997; Teupser et al., 1999]. Briefly PBMC pellet was resuspended in 200 µl ice-cold buffer A [10 mM HEPES pH 7.9, containing 10 mM KCl, 0.1 mM EDTA, 1.5 mM $MgCl_2$, 1 mM Dithiothreitol (DTT), 1 mM Phenyl- methylsulfonyl-fluoride (PMSF), 1% Protease inhibitor Cocktail and 0.4 % IGEPAL (Sigma, Germany)]. Protease inhibitor cocktail contained in 1 ml: 500 µg Antipain, 500 µg Aprotinin, 500 µg Leupeptin, 50 µg Pepstatin, 750 µg Bestatin, 400 µg Phosphoramidon and 500 µg Trypsin inhibitor (all obtained from Roche Mannheim, Germany). Cell suspension was incubated on ice for 10 minutes in order to lyse the cells and then centrifuged at 12,000 g for 5 minutes (4°C). Pellet was resuspended in buffer A and recentrifuged. The nuclear pellet so obtained was suspended in 100 µl buffer B (20 mM HEPES pH 7.9, containing 0.4 M NaCl, 1 mM EDTA, 1 mM DTT, 1mM PMSF and 1% protease inhibitor cocktail). After centrifugation at 12,000 g for 5 minutes (4°C), supernatant (nuclear extract) was frozen at -80°C.
For nuclear extracts from placenta approximately 3g of placental tissue was placed in 1ml ice-cold buffer A (10 mM HEPES pH 7.6, 0.1 mM EDTA, 15 mM KCl, 2 mM $MgCl_2$, 0.2% Igepal) and minced with surgical knife, homogenised (Polytron, Kinematica AG) and centrifuged at 850 g at 4°C for 5 minutes. Pellet was washed once with 1 ml buffer A, resuspended in 1 ml of buffer B (10 mM HEPES pH 7.6, containing 0.25 M sucrose, 0.1 mM EDTA, 15 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.5 mM PMSF, 0.3% Leupeptin) and centrifuged at 850 g for 10 minutes. It was then resuspended in 100µl of buffer C (50 mM HEPES pH 7.9, containing 0.1 mM EDTA, 400 mM KCl, 10% Glycerol, 1 mM DTT, 1 mM PMSF, 0.3% Leupeptin), shaken at 4°C for 30 minutes, centrifuged again at 12,000 g for 10 minutes. Supernate containing nuclear extract was aliquoted and stored at -80°C together with buffer C.

2.8.1. DNA binding assay for NF-kappa B:

Consensus oligonucleotides 5'-AGT-TGA-GGG-GAC-TTT- CCC-AGG-C- 3' and 5'-GCC- TGG-GAA-AGT-CCC-CTC-3' (Eurogentec Belgium) were annealed and labeled with [α-^{32}P]dATP (Amersham, Germany) in the presence of deoxy- nucleoside triphosphates by primer extension with the Klenow fragment of DNA polymerase I (Roche Mannheim, Germany). Nuclear proteins were incubated with ^{32}P labeled oligonucleo- tide in binding buffer (5 mM HEPES pH7.9, containing 0.12 mg/ml Poly [d(I-C)], 2.5 mg/ml BSA, 20% glycerol, 25 mM KCl, 2.5 mM EDTA, 125 mM NaCl, 25 mM Tris-HCl buffer pH 7.5, 35 mM DTT, 5 mM PMSF, 0.1 mg/ml Aprotinin, and 0.25% Igepal) for 15-30 minutes. DNA bound NF-κB was subjected to eletrophoresis on 4% acrylamide/bisacrylamide gel in 0.25x Tris-borate-EDTA buffer (4°C) until the tracer dye reached 60% of gel length. Approximately 12 μg nuclear protein for placental tissue and 4.5μg nuclear protein for PBMC's were applied to each lane. Gels were dried, exposed to storage phosphor screens, read in a phosphorimager (Molecular Dynamics, Krefeld Germany) and quantified using Image Quant software. Nuclear extract from THP-1 cells stimulated by lipopolysaccharide (LPS) were used as positive controls.

2.8.2. *DNA binding assay for AP-1:*

Double-stranded activator protein-1 (AP-1) consensus oligonucleotide was labeled with [γ-^{32}P]ATP (Amersham) using T4 polynucleotide kinase and purified on ProbeQuant™ G50 microcolumns (Amersham, Pharmacia). For DNA binding assays, nuclear proteins were incubated with binding buffer on ice for 10 minutes (25 mM Tris-HCl pH 7.5, containing 10% glycerol, 2.5 mM $MgCl_2$, 1.25 mM EDTA, 0.5 mM DTT, 125 mM NaCl, and 0.125 mg/ml Poly [d(I-C)]). The radiolabeled AP-1 probe (50,000 cpm/μl) was then added and the mixture was incubated on ice for another 30 minutes. After addition of 2 μl of 50% Glycerol, samples were loaded on a nondenaturing 5% acrylamide/bisacrylamide (29:1) gel. DNA protein complexes were separated by electrophoresis at 20V (4°C) for 16 hours in 0.25x Tris-borate-EDTA buffer. Protein content of nuclear extract applied to each

lane was 2µg and 2.7µg for placental tissue and PBMC respectively. Gels were dried, exposed to storage phosphor screens, and quantified by phosphorimage analysis.

2.9. Analytical techniques:

The protein content of nuclear extracts from PBMC and placental tissue as well as lipoproteins was measured by the method of Lowry et al (1951). The concentrations of cholesterol, phospholipids, and triglycerides were determined by standard enzyme kit methods (Lipidophor technique, Immuno A.G., Vienna, Austria).
Quantitative measurement of sVCAM-1, sICAM-1, sE-selectin, Endothelin-1, and MCP-1 were performed by means of solid phase sandwich enzyme-linked immunosorbent assay (ELISA) (R&D, Minneapolis, USA), with ranges of sensitivity < 2.0 ng/ml, <0.35 ng/ml, <0.1 ng/ml, <1.0 ng/ml and <5.0 ng/ml respectively. TNF-α was measured by solid phase enzyme amplified sensitivity immunoassay (EASIA) (Biosource, Europe S.A.) with sensitivity range of 3 pg/ml. Serum LBP was measured by means of chemiluminescence sandwich immuno- assay on IMMULITE® (Diagnostic Products Corporation, USA) with calibration range up to 200 µg/ml and sensitivity range of 0.2 µg/ml.
Plasma homocysteine was measured by fluorescence polarization immunoassay (FPIA) on the IMx® immunoassay system (Abbott, USA) with sensitivity range of <0.5 µmol/L. Tissue factor was measured in sodium citrate plasma by IMUBIND® ELISA kit (Loxo GmbH, Dossenheim, Germany) with detection limit of 10 pg/ml.
Possible hemodilution for serum and plasma markers after the H.E.L.P.-apheresis was corrected by hematocrit (HCT) as follows: $\text{Value}_{\text{corrected}} = \text{Value}_{\text{measured}} \times (1 - \text{HCT}_{\text{after}})/(1 - \text{HCT}_{\text{before}})$.

2.10. Statistical analysis:

All the measured parameters are presented as mean ± standard deviation. Statistical significance was evaluated by Wilcoxon's test between paired samples using the SAS software (version 8.2, SAS Institute Inc, Cary, NC). For all analyses, $P < 0.05$ was considered significant.

3. Results

3.1. Modulation of proatherogenic factors by H.E.L.P. therapy:

Proatherogenic lipoproteins LDL, Lp(a), and procoagulatory factors such as fibrinogen, tissue factor and homocysteine play a major role in atherosclerosis. Various inflammatory markers including cell adhesion molecules (VCAM-1, ICAM-1, and E-selectin), monocyte chemoattractant protein-1 (MCP-1), endothelin-1 (ET-1) and CRP alter the function of endothelium. Since preeclampsia mainly arises from endothelial dysfunction, we wanted to investigate whether in addition to reducing LDL, Lp(a), and fibrinogen, H.E.L.P. therapy would also reduce proinflammatory markers. For this reason, a preliminary study was undertaken on CHD patients who are regularly undergoing H.E.L.P. therapy.

As was to be expected, single H.E.L.P. therapy decreased plasma total cholesterol, LDL-C, VLDL-C, Lp(a), and triglycerides by 50%, 61%, 39%, 62%, and 53% respectively ($P < 0.001$) (Table 1).

Table 1. Effect of single H.E.L.P.-apheresis on plasma lipids and lipoproteins.

Lipids (mg/dl)	Pre-HELP	Post-HELP	Reduction (%)	P value
Total cholesterol	216.1±38.1	107.0±20.4	50.3±5.9	<0.001
Triglycerides	232.7±178.8	111.0±100.4	53.3±18.7	<0.001
LDL-cholesterol	128.6±29.9	49.8±17.1	61.4±9.3	<0.001
HDL-cholesterol	47.6±14.4	37.8±8.6	17.0±22.2	<0.001
VLDL-cholesterol	36.8±33.1	17.3±11.3	38.5±54.5	<0.001
Lp(a)	91.3±48.2	32.5±18.0	61.7±20.6	<0.001
Apo AI	150.8±27.3	119.2±26.0	21.3±5.7	<0.001
Apo B	109.2±23.6	42.1±12.3	61.8±6.0	<0.001

An interesting observation was that H.E.L.P. therapy reduced proinflammatory parameters, such as sVCAM-1, sE-selectin MCP-1, ET-1, LBP, and hs-CRP by 37%, 26%, 15%, 24%, 27%, and 67% respectively (Table 2). These changes were statistically significant ($P < 0.01$).

Table 2. Modulation of serum and plasma proinflammatory markers by a single H.E.L.P.-apheresis.

Proinflammatory Parameters	Pre-HELP	Post-HELP	Reduction (%)	P value
sVCAM-1 (ng/ml)	674.8±185.8	426.5±151.5	37.0±10.9	<0.001
sICAM-1 (ng/ml)	148.1±37.5	153.9±40.9	-6.2±20.4	0.427
sE-selectin (ng/ml)	39.4±37.6	24.7±10.3	25.9±16.7	0.045
MCP-1 (pg/ml)	409.9±124.2	346.9±110.0	15.0±11.1	<0.001
ET-1 (pg/ml)	1.8±0.5	1.3±0.3	24.4±15.9	<0.001
LBP (μg/ml)	7.1±3.8	4.7±2.1	26.7±31.3	<0.01
Hs-CRP (mg/dl)	0.50±1.24	0.17±0.4	66.9±15.7	<0.001

sVCAM-1: soluble vascular cell adhesion molecule. sE-selectin: soluble endothelial selectin. MCP-1: monocyte chemoattractant protein-1. ET-1: endothelin-1. LBP: lipopolysaccharide binding protein. Hs-CRP: high sensitive CRP.

Single H.E.L.P. therapy also reduced fibrinogen by 66% as was to be expected. In addition we noted reductions in tissue factor (TF) and homocysteine by 27% and 22% respectively (Table 3).

Table 3. Modulation of procoagulatory factors by a single H.E.L.P.-apheresis.

Procoagulatory parameters	Pre-HELP	Post-HELP	Reductio n (%)	P value
Tissue factor (pg/ml)	280.0±118.4	211.4±113.8	26.5±37.4	<0.001
Fibrinogen (mg/dl)	295.6±72.3	100.9±38.1	66.1±7.0	<0.001
Homocysteine (μmol/L)	12.3±4.4	9.6±3.3	21.6±4.9	<0.001

sCD40L: soluble CD40 ligand.

3.2. Plasma lipids, lipoproteins and proinflmmatory markers in normal pregnant women and preeclamptic patients:

Clinical studies have shown increased levels of total cholesterol, triglycerides, LDL-C, VLDL-C, and FFA in normal pregnant women [Knopp et al., 1973, 1984, 1986; Desoye et al., 1987; Steitz et al., 1987; Metzger, 1982]. These alterations are more pronounced in preeclampsia [Potter and Nestel, 1979; Murai et al., 1997; Thadhani et al., 1998]. In contrast, no significant differences in lipid parameters,

homocysteine and vitamin E between normal pregnant women and preeclamptic patients were noted in the present study (Table 4).

Table 4. Comparison of lipids and lipoproteins between normal pregnant women and preeclamptic patients.

Lipids (mg/dl)	Normal pregnancy n=13	Preeclampsia n=6	P Value
Total cholesterol	217±49 (125-327)	215±50 (163-276)	NS
Phospolipids	233±48 (143-317)	212±26 (172-237)	NS
Triglycerides	196±43 (96-264)	151±57 (63-202)	NS
LDL-cholesterol	137±41 (66-212)	124±34 (75-167)	NS
HDL-cholesterol	59±15 (35-89)	64±14 (38-79)	NS
VLDL-cholesterol	27±9 (15-45)	27±22 (5-66)	NS
Apo AI	170±25 (121-210)	178±37 (120-222)	NS
Apo B	112±27 (55-166)	103±26 (73-139)	NS
Lp(a)	27±20 (0-74)	29±29 (11-87)	NS
LDL/HDL ratio	2.3±0.5 (1.5-3.5)	2.1±0.9 (1.1-3.7)	NS
FFA (mmol/l)	0.96±0.21(0.57-1.3)	1.2±0.5 (0.5-1.7)	NS
Hcy (μmol/l)	5.3±1.5 (3.3-8.3)	6±1.4 (4.2-7.7)	NS
Vitamin E (mg/l)	21±8 (13-40)	20±11 (11-39)	NS

Figures in Brackets denote range. Hcy: homocysteine. NS: no statistically significant differences.

There was a slight but significant reduction of triglycerides and increase in cholesterol content in VLDL fractions of preeclamptic patients (Table 5).

Table 5. Comparison of lipid-protein composition of lipoproteins isolated from plasma of normal pregnant women and preeclamptic patients.

Lipop-rotein	Lipid-protein content (%)	Normal pregnancy n=12	Preeclampsia n=6	P value
VLDL	Cholesterol	14±2 (11-17)	17±4 (9-21)	0.050
	Phospholipids	15±2 (10-18)	15±4 (9-20)	NS
	Triglycerides	53±7 (42-63)	44±14(30-70)	0.044
	Protein	18±5 (11-25)	25±9 (13-39)	NS
LDL	Cholesterol	34±5 (22-41)	35±2 (33-39)	NS
	Phospholipids	23±1 (20-25)	23±1 (22-25)	NS
	Triglycerides	14±4 (8-19)	13±2 (11-16)	NS
	Protein	29±3 (25-35)	29±2 (26-32)	NS
HDL	Cholesterol	15±2 (9-18)	16±1 (14-17)	NS
	Phospholipids	25±4 (17-32)	28±3 (24-32)	NS
	Triglycerides	7±3 (2-13)	6±1 (4-8)	NS
	Protein	53±6 (48-69)	51±2 (48-54)	NS

Values are given as percentage (%). Figures in Brackets denote range. NS: no statistically significant differences.

Except for TNF-R75 levels which were higher in preeclamptic patients (P < 0.05) compared to normal pregnant women, no significant differences in other proinflammatory markers between the two groups were noted (Table 6).

Table 6. Comparison of circulating proinflammatory parameters in normal pregnant women and preeclamptic patients.

Proinflammatory parameters	Normal pregnancy n=12	Preeclampsia n=6	P value
TNF-R55 (ng/ml)	2.3±1.1 (1.4-4.9)	2.4±0.5 (1.6-3.1)	NS
TNF-R75 (ng/ml)	3.8±0.8 (2.6-5.3)	5.4±1.9 (3.9-8.8)	0.044
IL-6 (pg/ml)	5.7±2.9 (2.5-13.1)	11.1±13.3 (2.8-37.8)	NS
LBP (μg/ml)	7.5±2.3 (4.1-11.3)	10.0±6.4 (4.8-22.3)	NS
sICAM-1 (ng/ml)	178±74 (77-292)	183±57 (125-251)	NS
sVCAM-1 (ng/ml)	580±144 (449-867)	731±277(414-1064)	NS
Fibrinogen (mg/dl)	307±154(171-531)	440±155 (209-609)	NS

Figures in Brackets denote range. NS: no statistically significant differences.

3.3. Circulating levels of lipids, lipoproteins and proinflammatory markers in placental blood from normal pregnant women and preeclamptic patients:

Preeclampsia results in profound changes in placenta, which include placental ischemia, lipoprotein toxicity, immune maladaption and endothelial dysfunction. It was therefore necessary to examine if a correlation between maternal and fetal components exists. We measured circulating levels of lipids, lipoproteins, lipid-protein composition of lipoprotein subfractions, proinflammatory and procoagulatory factors. No significant alterations in various lipids and lipoproteins were noted between the two groups except for lower levels of triglycerides, Lp(a) and higher content vitamin E in the placental serum from preeclamptic patients (Table 7).

Table 7. Comparison of lipids and lipoproteins in placental blood from normal pregnant women and preeclamptic patients.

Lipids (mg/dl)	Normal pregnancy n=41	Preeclampsia n=5	P value
Total cholesterol	75±22 (42-123)	77±32 (38-112)	NS
Phospholipids	121±23 (77-177)	124±26 (92-153)	NS
Triglycerides	28±18 (14-119)	43±18 (21-64)	0.046
LDL-cholesterol	31±13 (13-72)	46±28 (17-85)	NS
HDL-cholesterol	34±11 (16-56)	24±11 (15-40)	NS
VLDL-cholesterol	9±6 (1-29)	8±4 (4-14)	NS
Apo AI	73±12 (48-92)	64±14 (47-81)	NS
Apo B	27±11 (11-70)	33±12 (23-50)	NS
Lp(a)	6±5 (0-18)	2.3±4.5 (0-9)	0.037
LDL/HDL ratio	0.9±0.4 (0.4-2.5)	1.8±0.9 (1.0-2.9)	0.003
FFA (mmol/l)	0.33±0.14 (0.14-0.71)	0.23±0.03 (0.18-0.26)	NS
Hcy (μmol/l)	6±1.7 (4-11.5)	4.9±3.1 (3.1-9.5)	NS
Vitamin E (mg/l)	5.3±1.8 (3.2-9.1)	10.2±4.4(4.1-14)	0.050

Figures in Brackets denote range. Hcy: homocysteine. NS: no statistically significant differences.

No major alterations in the lipid-protein composition of isolated lipoprotein fractions from the plasma of placenta from normal pregnant women and preeclamptic patients were noted (Table 8).

Table 8. Comparison of lipid-protein composition of isolated placental plasma lipoproteins from normal pregnant women and preeclamptic patients.

Lipop-rotein	Lipid-protein content (%)	Normal pregnancy n=39	Preeclampsia n=5	P Value
VLDL	Cholesterol	17±11 (3-53)	19±4 (12-23)	0.050
	Phospholipids	7±5 (0.1-18)	16±5 (8-19)	NS
	Triglycerides	60±16 (36-93)	43±15 (32-70)	0.020
	Protein	15±9 (3-54)	23±8 (11-32)	0.030
LDL	Cholesterol	31±5 (16-40)	32±5 (24-37)	NS
	Phospholipids	21±4 (10-30)	21±4 (15-26)	NS
	Triglycerides	21±10 (4-53)	20±13 (11-44)	NS
	Protein	27±5 (19-33)	26±5 (18-30)	NS
HDL	Cholesterol	16±3 (10-22)	17±1 (16-19)	NS
	Phospholipids	27±3 (19-33)	29±3 (26-33)	NS
	Triglycerides	6±4 (2-21)	5±2 (2-6)	NS
	Protein	52±3 (46-58)	49±2 (47-52)	0.048

Values are given as percentage (%). Figures in Brackets denote range. NS: no statistically significant differences.

Similarly, levels of circulating proinflammatory markers remained unchanged except for an increase of IL-6 levels in placental serum from preeclamptic patients (Table 9).

Table 9. Comparison of placental circulating proinflammatory parameters in normal pregnant women and preeclamptic patients.

Proinflammatory parameters	Normal pregnancy	n	Preeclampsia	n	P value
TNF-R55 (ng/ml)	6.2±2.6 (3.3-15.6)	35	5.3±0.6 (4.8-6)	5	NS
TNF-R75 (ng/ml)	8.4±1.4 (5.5-11.7)	37	7.3±1.3 (6-9)	5	NS
IL-6 (pg/ml)	16±11 (3-50)	37	36±24 (20-72)	5	0.024
LBP (μg/ml)	2.7±1.7 (0.7-9.9)	26	1.7±0.8 (0.8-2.7)	5	NS

Figures in Brackets denote range. NS: no statistically significant differences.

3.4. Ameliorative effects of H.E.L.P.-apheresis in preeclamptic patients:

Preeclampsia is accompanied by alterations in lipoprotein profile, disturbance in coagulatory function and increased levels of proinflammatory markers. H.E.L.P. therapy effectively reduces the concentrations of LDL, Lp(a), fibrinogen and improves the blood viscosity as well as coagulatory functions (Walzl et al., 1993; Schuff-Werner and Holdt, 2002; Schuff-Werner et al., 1989). In our preliminary study on CHD patients (Table 1-3), it was shown that in addition to reducing lipoproteins and fibrinogen, H.E.L.P. therapy also reduced the levels of circulating proinflammatory markers. Since long-term application of H.E.L.P. therapy established that this procedure is safe and without any serious adverse events, three preeclamptic patients were treated with this therapy. Both the clinical situation as well as fibrinogen levels were taken as criteria for the number of therapies in these patients. We also included other 3 preeclamptic patients during this study who were either unwilling to participate in H.E.L.P. therapy or underwent cesarean section because of progressive preeclampsia as control group to the treated patients. All these 6 patients underwent cesarean sections. Delivery was initiated either because of progressive preeclampsia or because of a nonreassuring fetal status with a pathologic cardiotocography (CTG) or placental color Doppler findings. All newborns were treated in an adjacent facility with specialized neonatal support. Clinical information of 6 preeclamptic patients either on H.E.L.P. therapy or without this therapy are given in Table 10 (a-c). For the sake of clarity each patient is described.

Table 10a. Clinical characteristic of preeclamptic patients.

	Patients on H.E.L.P. therapy			Patients without H.E.L.P.therapy		
	Nr.1	Nr.2	Nr.3	Nr.4	Nr.5	Nr.6
Age (years)	24	34	33	23	36	37
Ethnic descent	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Body weight (kg)	76	126	95	73	70	62
Gravida	1	2	1	1	1	1
Para	1	2	1	1	1	1
Gestational age at admission (weeks)	24^{+1}*	26^{+2}	27^{+3}	31^{+6}	23^{+5}	28^{+6}
Systolic BP (mmHg)	156	140	140	150	145	130
Diastolic BP (mmHg)	108	90	80	110	90	85
Number of HELP therapies	3	6	4	--	--	--
Gestational age at cesarean delivery (weeks)	25	29^{+4}	29^{+6}	31^{+6}	23^{+5}	28^{+6}

$^{+}$ denotes days.

Table 10b. Laboratory parameters of preeclamptic patients.

	Patients on H.E.L.P. therapy			Patients without H.E.L.P.therapy		
	Nr.1	Nr.2	Nr.3	Nr.4	Nr.5	Nr.6
Blood						
Hemoglobin (g/dl)	13.0	13.2	12.2	10.7	11.0	13.9
Hematocrit	0.388	0.378	0.353	0.350	0.301	0.408
GOT (25º) (reference 5-15 U/l)	7	20	21	14	622	45
GPT (25º) (reference 5-19 U/l)	10	19	19	13	424	77
LDH (25º) (reference 80-240 U/l)	161	187	190	232	986	356
Creatinine (mg/dl)	0.8	0.7	0.7	0.8	0.9	0.6
Urea (mg/dl)	15	23	22	17	40	22
Total protein (g/dl)	5.2	5.7	5.4	5.1	5.3	6.3
Albumin (g/dl)	3.6	3.7	3.4	2.7	3.4	3.6
Urine						
pH (reference 5-6)	5	5	5	8	5	5
Albumin (g/l)	1	0.3	0.3	5	0.3	0.3

Table 10c. Doppler ultrasound and cardiotocography findings of preeclamptic patients.

	Patients on H.E.L.P. therapy			Patients without H.E.L.P.therapy		
	Nr.1	Nr.2	Nr.3	Nr.4	Nr.5	Nr.6
Doppler ultrasound						
UA						
RI	Reverse end-diastolic flow	0.83	Zero end-diastolic flow	0.67	0.83	-
MCA						
RI	0.73	0.83	0.87	-	-	-
Cardiotocography	-	-	-	Pathologic	Pathologic	Intermittent asystole

UA: umbilical artery. MCA: middle cerebral artery. RI: resistance index.

3.4.1. Case reports:

Patient 1: A 24-year-old woman (gestational age 24^{+1} weeks) was referred to Klinikum Grosshadern on 30th Nov 2002.
Since 9 days she had increased blood pressure values, generalized edema without preexisting history of hypertension or vascular disease before pregnancy. Blood pressure could not be satisfactorily controlled with routine antihypertensive therapy. Both her mother and sister had preeclampsia during their first pregnancy resulting in preterm deliveries.
Physical examination revealed clear mental status, generalized edema, and elevated blood pressure (156/108 mmHg). No signs of neurological disorders were evident.
Patient had hypoalbuminemia (albumin 3.1 g/dl) and proteinuria. Her serum total cholesterol, LDL-C, VLDL-C, HDL-C, and Lp(a) levels were 167, 93, 11, and 16 mg/dl respectively. Triglycerides and fibrinogen levels were within normal range (110 and 334 mg/dl respectively).
Placental Doppler findings showed singleton fetus with an estimated fetal weight of 400 g (< 10th percentile of normal range) and reverse blood flow of umbilical artery (UA), establishing preeclampsia with intrauterine growth retardation (IUGR).
After placing a double-lumened central venous catheter, she was treated with H.E.L.P.-apheresis within 2 hours after admission to

hospital. Therapy was repeated on 2nd and 5th day of hospitalization. No adverse events were observed during the H.E.L.P. except a flush after first therapy possibly due to the improved blood viscosity and hyperemia. Placental Doppler showed improved flow in uterine artery, fetal umbilical artery and middle cerebral artery (MCA).
The patient underwent cesarean section delivery after 3rd H.E.L.P. therapy because of urinary tract infection on 6th day. She delivered a female child weighing 400 g with Apagar score 7/9 at 1'/5' minutes. Her umbilical arterial blood pH was 7.24. The baby was kept in NICU (neonatal intensive care unit) on artificial ventilation for 7 weeks. No complications were evident and baby showed normal growth and weight gain. The mother had an uneventful postpartum stage. Antihypertension medicine was stopped 2 months after delivery when the blood pressure returned to normal value.

Patient 2: A 34-year-old woman (gestational age 26^{+2} weeks) was admitted to Klinikum Grosshadern on 30th April 2003.
She had increased blood pressure (systolic 140 mmHg; diastolic 90 mmHg) without day-night difference since 5 days. Her first pregnancy was complicated with preeclampisa and she underwent cesarean delivery in the 28th week of gestation. History of preeclampsia in the family is unknown.
She had normal mental and neurological status but generalized edema. Her serum total cholesterol, triglycerides, LDL-C, HDL-C, VLDL-C, and Lp(a) levels were 239, 213, 160, 44, 35, 22 mg/dl respectively. She had elevated levels of fibrinogen (490 mg/dl) and hypoalbuminemia (3.7 g/dl).
Placenta Doppler and CTG findings revealed abnormal flow rates in umbilical artery, oligohydramnion, small fetus with estimated fetal weight of 730g, establishing preeclampsia with intrauterine growth retardation.
First H.E.L.P. therapy was performed on the 3rd day after hospitalization. Thereafter 5 more treatments followed on 5th, 8th, 9th, 12th, and 22th day of hospitalization. Placenta Doppler examinations were done after each treatment. CTG and blood pressure were monitored during the whole therapy. Modulation of placentofetal blood flow resistance by H.E.L.P. therapy in this patient was shown in Table 11.

Table 11. Modulation of placentofetal blood flow resistance by H.E.L.P. therapy in patient Nr.2.

Doppler ultrasound findings	At admission	After 2 therapies*	After 3 therapies*	After 4 therapies*	On day of CS
RI (UA)	0.83	0.61	0.72	0.80	Zero end-diastolic flow
RI (MCA)	0.83	--	--	0.73	0.81
Hospitalization day	1st	5th	8th	9th	23th

*: Doppler ultrasound examinations were performed within several hours after each H.E.L.P. therapy. CS: cesarean section.

Patient remained clinically stable. It was decided to deliver the baby by cesarean section at the gestational age of 29^{+4} weeks. She delivered a male child weighing 870 g, pH value of umbilical arterial blood 7.32, with Apagar score 9/9/9/10 at 1’/5’/10’/20’ minutes respectively. As in the case of the first patient, baby was transferred to NICU. Both the baby and mother remained clinically stable. The baby was kept on artificial ventilation for 1 week. No complications were evident and baby showed normal growth and weight gain.

Patient 3: A 33-year-old woman who had undergone in vitro fertilization was referred to us on 7th May 2003 at the gestational age of 27^{+3} weeks. Her blood pressure had increased to 140/80 mmHg from a baseline level of < 120/80 mmHg within 2 days. Her mother had also suffered from preeclampsia during first pregnancy and patient herself was a pre-term baby.

On admission she had normal mental and neurological status, but generalized edema. Analysis of serum lipids revealed hyperlipidemia. Her total cholesterol, LDL-C, HDL-C and VLDL-C levels were 274, 167, 73, 34 mg/dl respectively. In addition, she had elevated levels of Lp(a) (87 mg/dl) and fibrinogen (609 mg/dl).

Placenta Doppler and CTG examinations revealed altered umbilical artery flow (end-diastolic flow zero) and estimated fetus weight of 678 g establishing preeclampsia accompanied by placental insufficiency and intrauterine growth retardation.

After placing central venous catheter, she was treated with H.E.L.P.-apheresis on 2nd day of admission and this procedure was repeated on days 8, 10, 16. On the basis of placenta Doppler, it was decided to deliver the baby at 29^{+6} weeks of gestational age. She delivered a female baby with body weight of 890 g, pH value of umbilical arterial blood 7.22, Apagar score 8/9/9/9 at 1'/5'/10'/20' minutes respectively. Baby was kept in NICU on artificial ventilation for 1 week. No complications were evident and baby showed normal growth and weight gain. The mother had an uneventful postpartum stage. After the first H.E.L.P. therapy, antihypertensive medication in this patient was stopped.

Clinical outcome of newborns from preeclamptic mothers without H.E.L.P. therapy: Clinical information about newborns from three preeclamptic patients who were not treated by H.E.L.P. therapy are shown in Table 12.

Table 12. Clinical outcome of newborns from preeclamptic patients not treated with H.E.L.P. therapy.

Newborn	Patient Nr.4	Patient Nr.5	Patient Nr.6
Gender	female	female	female
Gestational weeks at birth	31^{+6}	23^{+5}	28^{+6}
Birth weight (g)	1950	350	950
pH value of umbilical arterial blood	7.10	7.20	7.30
Apgar score at birth	8/10/10 (1´/5´/10´)	Died immediately after birth	6/6/7/8 (1´2´/5´/10´)
Duration of mechanical ventilation	no	--	3 weeks
Other complications at neonatal stage	Perinatal infection	--	Meconium obstruction requiring surgical operation

All the three mothers had an uneventful postpartum stage and their blood pressure values returned to normal range at discharge.

Additional medications for 6 preeclamptic patients: All these 6 patients was treated with antihypertensive drugs such as Metoprolol (50mg, twice a day) or α-Methyldopa (250mg, twice a day). Dihydralazin (25 mg, every 6 hours) were administrated when edema existed. Antihypertensive medications were stopped when blood pressure returned to normal value after delivery. All the 6 patients were treated with betamethasone (intramuscular) 12 mg on day of admission, and the same dose was repeated 24 hours later in patients Nr.1 to 3. Magnesium sulfate infusion was administrated in 4 patients (Nr.1,4,5 and 6) at a speed of 1.8 g/hour from the day of admission till 2 or 3 days after delivery.

3.4.2. Reduction of lipoproteins, fibrinogen and hs-CRP by H.E.L.P. therapy:

H.E.L.P. therapy effectively reduced circulating levels of total cholesterol, triglycerides, LDL-C, Lp(a), fibrinogen and hs-CRP by 35%, 45%, 46%, 53% and 56% respectively in all the three patients. After H.E.L.P. therapy a minor reduction in HDL and increase in unesterified fatty acids probably due to release of endothelial bound post-heparin lipoprotein lipase was noted. Data from a representative patient is shown in Table 13.

Table 13. Alterations in circulating lipids, fibrinogen and high sensitive CRP in a preeclamptic patient on H.E.L.P. therapy.

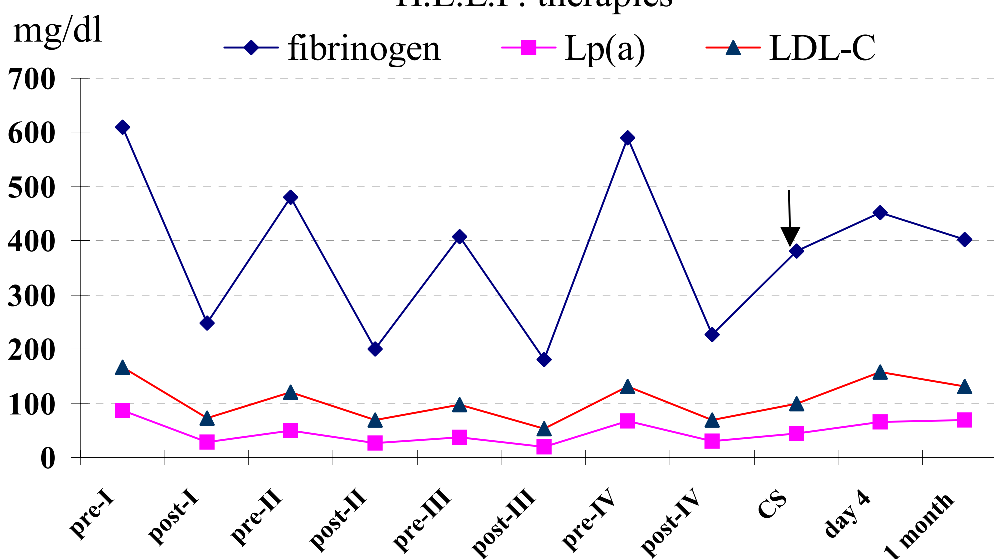
Parameters	**1st therapy**			**2nd therapy**			**3rd therapy**			**4th therapy**		
(mg/dl)	**Pre-**	**Post-**	**%▲**	**Pre-**	**Post-**	**%**	**Pre-**	**Post-**	**%**	**Pre-**	**Post-**	**%**
TC	274	144	-47	213	137	-36	185	114	-38	230	146	-37
Triglycerides	202	84	-58	243	122	-50	244	124	-49	218	130	-40
Phospholipids	225	176	-22	211	160	-24	240	172	-28	236	180	-24
LDL-C	167	72	-57	120	70	-42	98	54	-45	131	69	-47
VLDL-C	34	15	-56	31	16	-48	36	14	-61	39	18	-54
HDL-C	73	57	-22	62	51	-18	51	46	-10	60	59	-2
Apo AI	212	163	-23	193	160	-17	194	149	-23	185	159	-14

Apo B	**139**	**57**	**-59**	**108**	**55**	**-49**	**86**	**41**	**-52**	**109**	**58**	**-47**
Lp(a)	**87**	**28**	**-68**	**49**	**26**	**-47**	**37**	**19**	**-49**	**67**	**30**	**-55**
FFA (mmol/l)	**1.0**	**2.0**	**+100**	**1.3**	**2.4**	**+85**	**1.8**	**2.5**	**+39**	**1.1**	**2.3**	**+109**
Fibrinogen	**609**	**248**	**-59**	**480**	**200**	**-58**	**407**	**181**	**-56**	**591**	**226**	**-62**
hs-CRP	**0.8**	**0.4**	**-50**	**2.5**	**1.2**	**-52**	**1.9**	**0.9**	**-53**	**2.8**	**1.5**	**-46**

▲: % denotes changes, + increase, - decrease. Data presented is from patient Nr.3. Other two patients showed similar patterns.

A representative follow-up of LDL, Lp(a) and fibrinogen up to 1 month after cesarean section in a patient who was treated repeatedly by H.E.L.P. therapy before delivery are shown in Figure 1.

Figure 1. Follow-up of LDL-C, Lp(a), and fibrinogen levels in a representative patient treated with multiple H.E.L.P. therapies



Pre-I, II, III: before 1st, 2nd, 3rd H.E.L.P. therapy
Post-I, II, III: after 1st, 2nd, 3rd H.E.L.P. therapy
CS: cesarean section
Arrow indicates the day of cesarean section.

3.4.3. Proinflammatory markers:

H.E.L.P. therapy reduced the circulating levels of soluble TNF-receptors (TNF-R55, TNF-R75) and IL-6 on average by 12, 10 and 13% respectively. Adhesion molecule sVCAM-1 was reduced by 24% whereas levels of sICAM-1 remained unchanged or even increased. Acute phase markers, such as LBP and hs-CRP, were reduced by 16 and 49% respectively. ET-1, a very potent vascoconstrictor was also

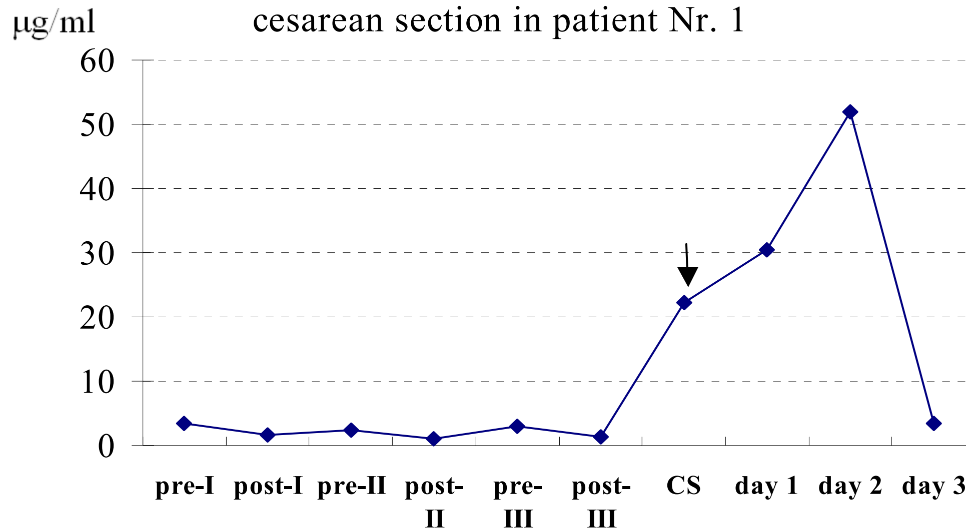
reduced drastically by single H.E.L.P. therapy. These reductions were observed in all the three patients undergoing H.E.L.P. therapy and could be observed also after repeated therapies (Table 14).
Since most pronounced changes were seen in hs-CRP and ET-1 representative data from one patient are shown in Figure 2a and 2b.

Table 14. Alterations of circulating inflammatory markers after H.E.L.P.- apheresis.

	1st therapy			2nd therapy			3rd therapy			Mean
Parameters	**Pre-**	**Post-**	**%▲**	**Pre-**	**Post-**	**%**	**Pre-**	**Post-**	**%**	**%**
TNF-α (pg/ml)	**28.4**	**12.2**	**-57**	**13.5**	**4.7**	**-65**	**20.7**	**39.1**	**+89**	**-11**
TNF-R55 (ng/ml)	**2.7**	**1.8**	**-33**	**1.7**	**1.9**	**+12**	**2.2**	**2.4**	**+9**	**-4**
TNF-R75 (ng/ml)	**5.9**	**4.3**	**-27**	**3.3**	**3.3**	**0**	**5.5**	**5.5**	**0**	**-9**
LBP (μg/ml)	**5.7**	**4.7**	**-18**	**4.7**	**5.0**	**+6**	**5.9**	**4.7**	**-20**	**-11**
hs-CRP (mg/dl)	**0.4**	**0.2**	**-50**	**0.2**	**0.1**	**-50**	**0.3**	**0.1**	**-67**	**-56**
sVCAM-1 (ng/ml)	**576**	**361**	**-37**	**411**	**288**	**-30**	**445**	**353**	**-21**	**-29**
sICAM-1 (ng/ml)	**125**	**118**	**-6**	**104**	**119**	**+14**	**95**	**128**	**+35**	**+14**
sE-selectin (ng/ml)	**19.9**	**11.9**	**-40**	**13.0**	**9.0**	**-31**	**12.5**	**13.2**	**+6**	**-22**
ET-1 (pg/ml)	**2.6**	**1.4**	**-46**	**0.6**	**0.1**	**-83**	**0.4**	**0.04**	**-90**	**-73**

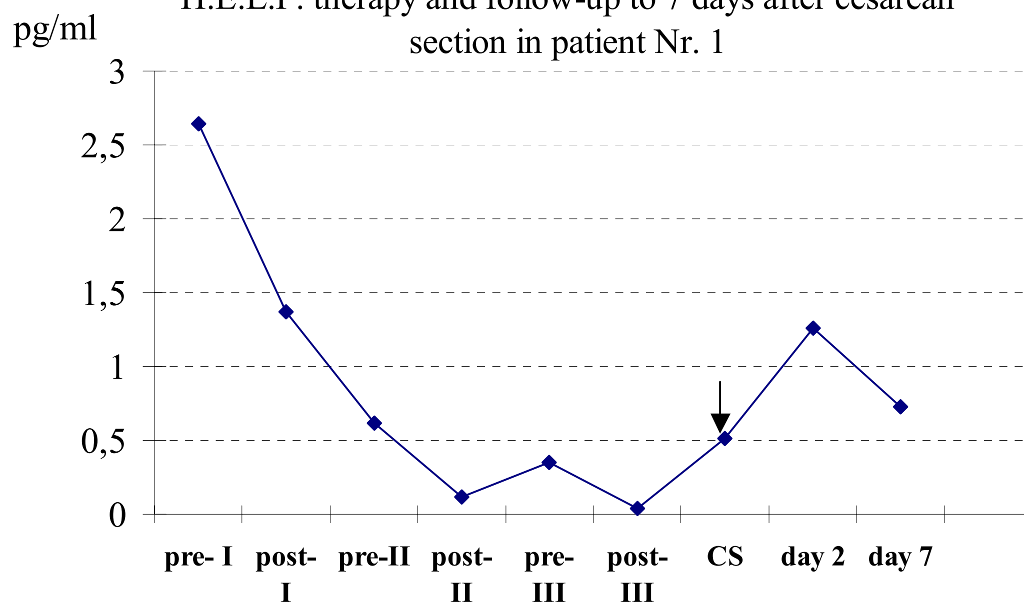
▲: % denotes changes, + increase, - decrease. Data presented is from patient Nr.1. Other two patients showed similar patterns.

Figure 2a. Reduction of serum hs-CRP levels by H.E.L.P. therapy and follow-up to 3 days after cesarean section in patient Nr. 1



Day 1, day 2, day 3 denote days after cesarean section.

Figure 2b. Reduction of plasma endothelin-1 levels by H.E.L.P. therapy and follow-up to 7 days after cesarean section in patient Nr. 1



Day 2, day 7 denote days after cesarean section.

3.4.4. Procoagulatory factors and plasma viscosity:

CD40-CD40 ligand (CD40L) is expressed in variety of cells including monocytes, dendritic cells, vascular smooth muscle cells, epithelial cells and thrombocytes. CD40L is membrane bound and its soluble form sCD40L arises from surface cleavage. Both cell membrane

bound and soluble CD40L interact with CD40 resulting in induction of tissue factor (TF), a major initiator in extrinsic coagulation cascade. Together with fibrinogen, they are procoagulant and thrombotic.
Next the effect of H.E.L.P. therapy on these factors was examined. On average single H.E.L.P. therapy reduced the circulating levels of fibrinogen, tissue factor and sCD40L by 53%, 30% and 34% respectively in all the three patients. This reduction of TF was also observed after repeated therapies (Figure3).

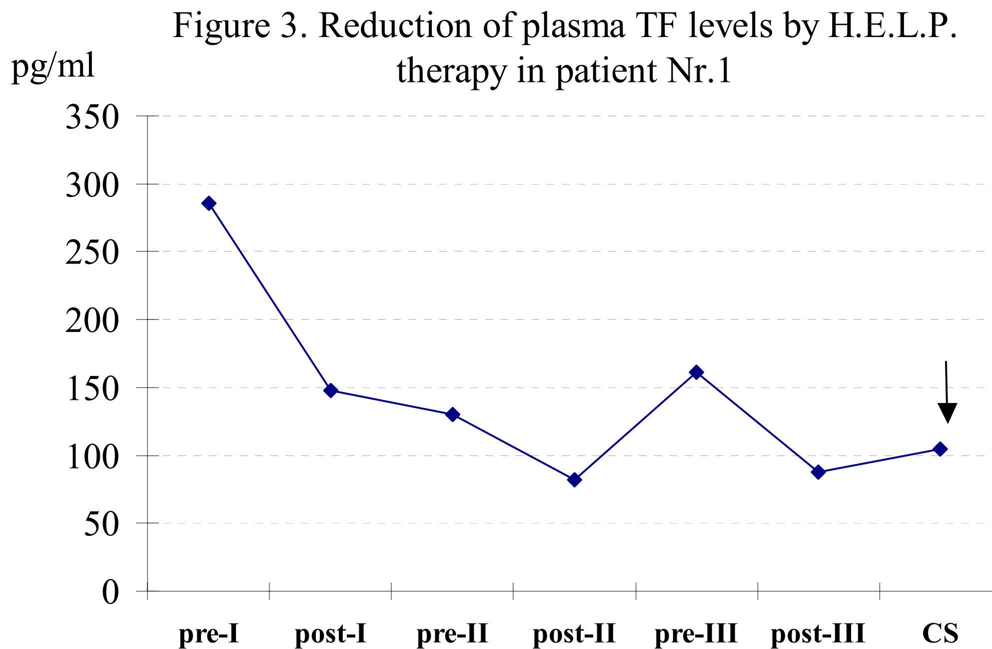
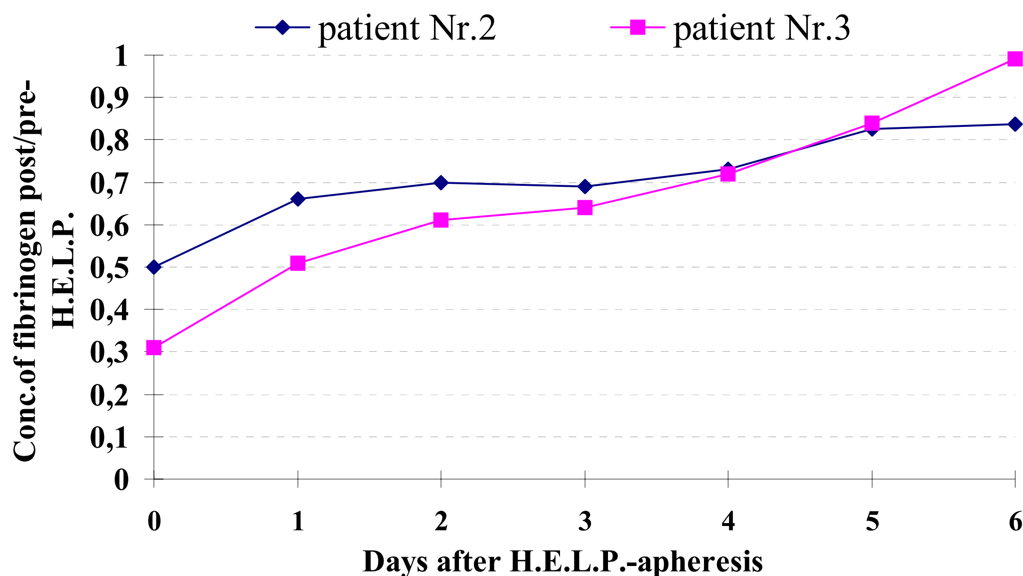


Figure 3. Reduction of plasma TF levels by H.E.L.P. therapy in patient Nr.1

3.4.5. Return of fibrinogen levels after H.E.L.P. therapy:

Since fibrinogen plays a major role in thrombotic process, it was of interest to study the kinetics of post-apheresis return of fibrinogen in order to calculate its synthetic and fractional catabolic rate. Plasma was collected immediately before and after H.E.L.P. therapy. During subsequent 7 days, blood samples were taken daily to follow return of fibrinogen. Fractional catabolic rate (FCR) and synthetic rates were calculated according to Apstein et al. (1978) and Grundy et al. (1985). Return of fibrinogen levels after a single apheresis in two patients is shown in Figure 4.



Calculation of turnover of fibrinogen is presented in Table 15. Fractional catabolic rate and synthetic rate in patient Nr.3 was 0.208 Pools•day^{-1} and 57.0 mg•kg^{-1}•day^{-1} respectively. Patient Nr.2 had lower turnover rates (0.165 Pools•day^{-1} and 36 mg•kg^{-1}•day^{-1}).

Table 15. Turnover data calculated from reaccumulation of fibrinogen (6 days) after H.E.L.P.-apheresis

	Patient Nr. 2	Patient Nr. 3
Fibrinogen level before H.E.L.P. (mg/dl)	490	609
Body weight	126	95
FCR (Pools·day^{-1})	0.165	0.208
Synthetic rate (mg·kg^{-1}·day^{-1})	36.4	57.0

The fractional catabolic rates (FCRs) for fibrinogen was calculated on the post-apheresis values according to Apstein (1978): $\{\ln [(Conc_o-Conc_t)/(Conc_o-Conc_{min})]=-kt\}$. $Conc_o$ is initial steady state concentration before H.E.L.P., $Conc_{min}$ is concentration directly after H.E.L.P. and $Conc_t$ is concentration at time ***t*** after cessation of H.E.L.P. Constant *k* is a first order disappearance constant. Least square regression through *–kt* of days after apheresis yields FCR of plasma protein. Plasma volume was taken as 4.5% of body weight.

The synthetic rate was estimated as the product of FCR and plasma pool size expressed per kilogram body weight (Grundy et al., 1985).

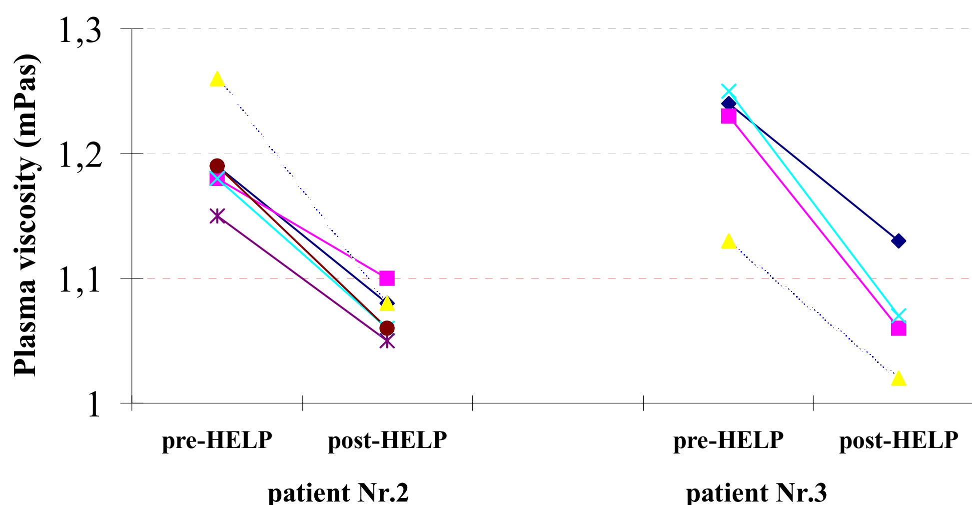
3.4.6. Plasma viscosity:

On average plasma viscosity was decreased by about 13% in all the three patients. Representative data from two patients are presented in Table 16 and Figure 5.

Table 16. Improvement of plasma viscosity by H.E.L.P.- apheresis in patient Nr.2 and Nr.3.

	Patient Nr.2							Patient Nr.3				
	1st	2nd	3rd	4th	5th	6th	mean	1st	2nd	3rd	4th	mean
Before	**1.19**	**1.18**	**1.26**	**1.18**	**1.15**	**1.19**		**1.24**	**1.23**	**1.13**	**1.25**	
After	**1.08**	**1.10**	**1.08**	**1.06**	**1.05**	**1.06**		**1.13**	**1.06**	**1.02**	**1.07**	
Reduction(%)	**-9.2**	**-6.8**	**-14.3**	**-10.2**	**-8.7**	**-10.9**	**-10.0**	**-8.9**	**-13.8**	**-9.7**	**-14.4**	**-11.7**

Figure 5. Improvement of plasma viscosity by single H.E.L.P.-apheresis in patient Nr.2 and Nr.3



3.4.7. Modulation of proinflammatory status in placental tissue:

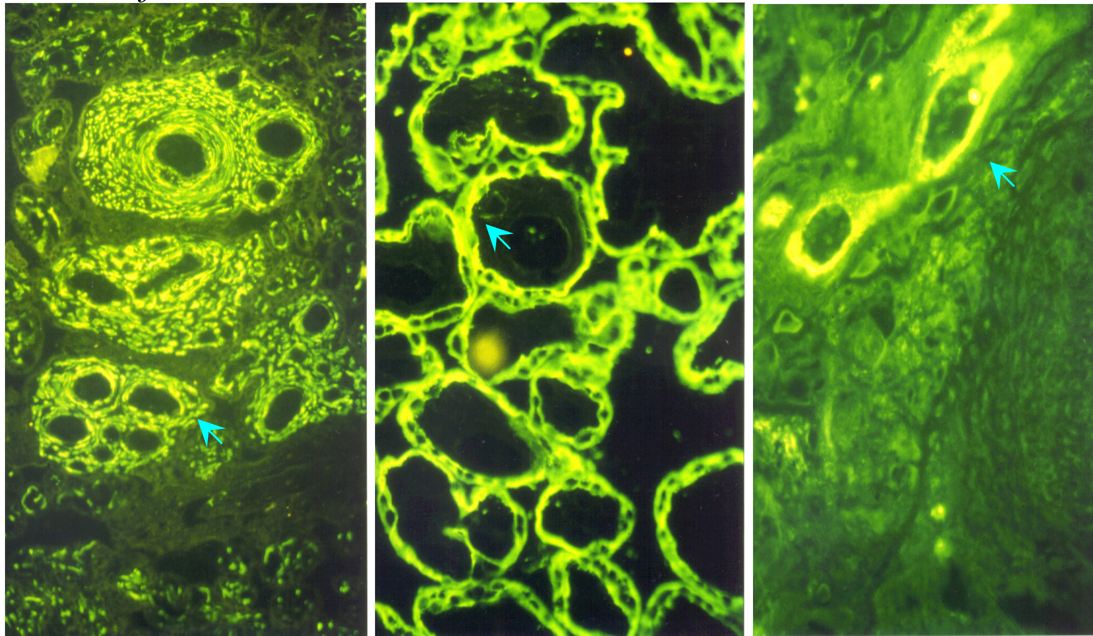
It has been postulated that in adequate remodeling of uterine spiral arteries in preeclampsia leads to focal ischemia and synthesis of proinflammtory cytokines by the placenta. One of earliest transcription factors which is now recognized to be ubiquitously expressed is NF-κB which can rapidly activate a number of target genes whose products are involved in inflammatory, immune and acute phase responses. Since preeclampsia either provokes or leads to endothelial dysfunction, endothelial NOS (eNOS) deserves attention. It was therefore of interest to examine both NF-κB and eNOS by immunocytoche- mistry.

3.4.7.1. Immunohistochemical studies:

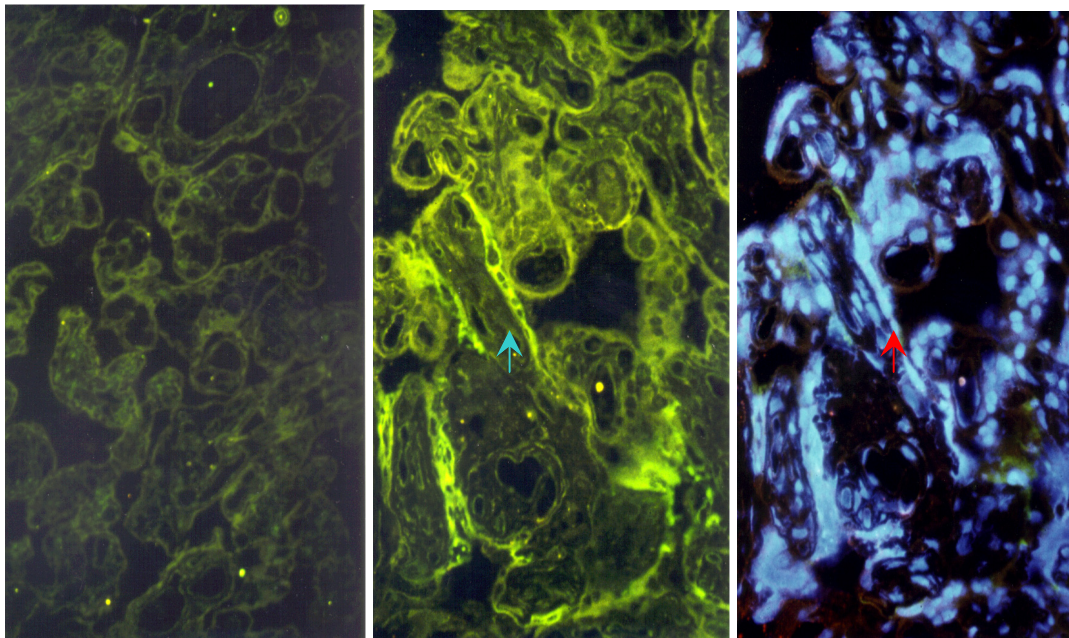
Placenta is a heterogenous organ comprising of various cells. Placental sections from normal pregnancy were stained by antibodies to cytokeratin, desmin and von Willebrand factor (factor VIII). Syncytiotrophoblasts mainly stained with cytokeratin (Figure 6a), whereas smooth muscle cells located in stem villi stained with desmin (Figure 6b). Von Willebrand factor was mainly located in endothelial cells in fetal vasculature (Figure 6c). In normal pregnant women (data not shown) and preeclamptic patients undergoing H.E.L.P.- apheresis, NF-κB was localized mainly in cytoplasm of syncytiotrophoblast (Figure 6e). In preeclamptic patients not undergoing H.E.L.P. therapy NF-κB was mainly localized in nuclei (Figure 6g).
eNOS was located mainly in the syncytiotrophoblast cells of placental villi in normal pregnant women and preeclamptic patients undergoing H.E.L.P. therapy (Figure 6i, 6j). In contrast, in patients not undergoing H.E.L.P. therapy it was present both in syncytiotrophoblast cells and fetal endothelial cells (Figure 6k).

Figure 6.

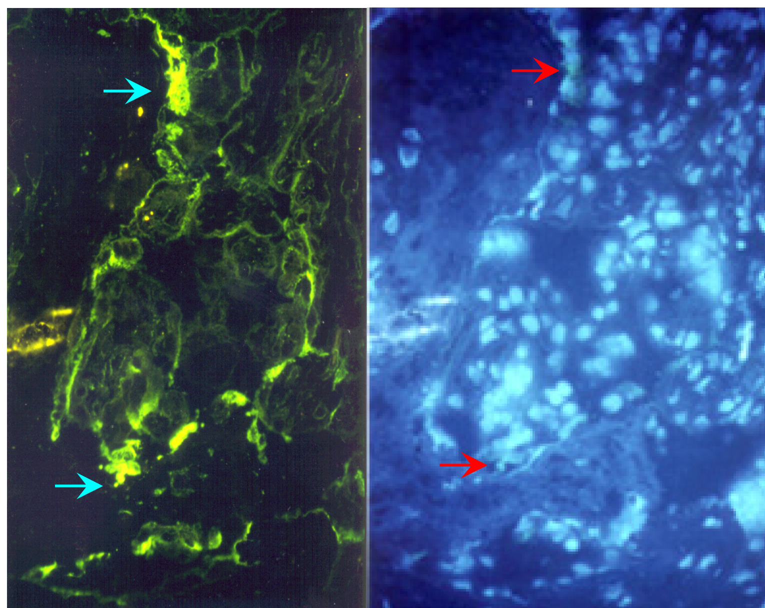
Immunofluroscence:



(a) (b) (c)

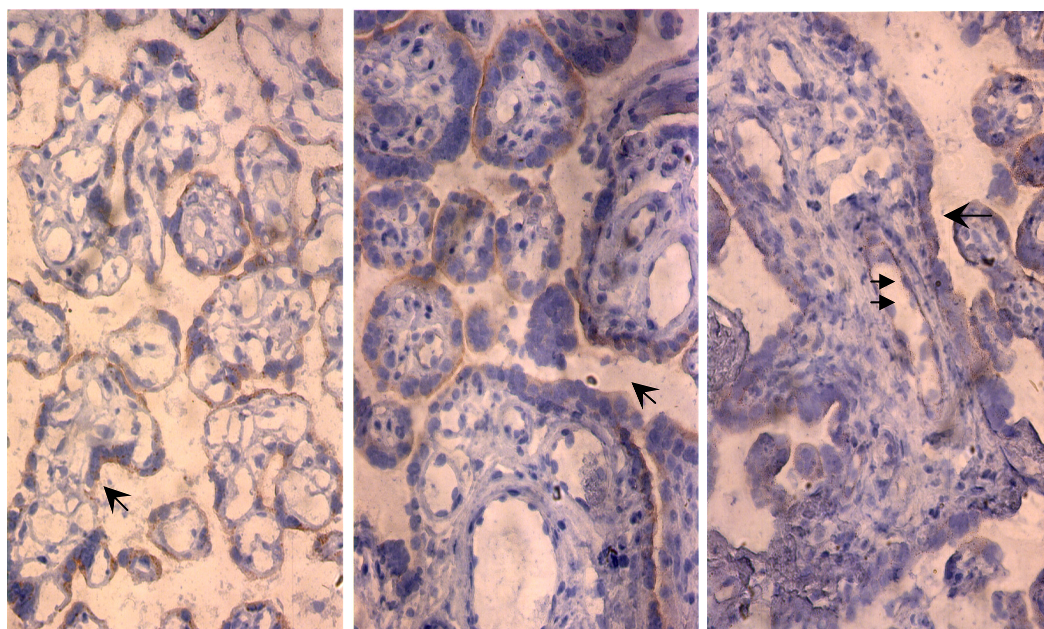


(d) (e) (f)



(g) (h)

Immunocytochemistry:



(i) (j) (k)

Characterization of placental villi by immunological reactions with antibodies to cytokeratin, desmin, and von Willebrand factor (factor VIII) (blue arrows) in placenta from normal pregnant women (a-c).

(a). Cytokeratin localized in syncytiotrophoblast cells.

(b). Desmin in smooth muscle cells of stem villi.

(c).Von Willebrand factor in endothelial cells of fetal vasculature. (d). Negative control.

(e-f):
(e). Cytoplasm localization of NF-κB in syncytiotrophoblast layer of patient on H.E.L.P. therapy (blue arrow).
(f). Nuclei stained by DAPI (red arrow).

(g-h):
(g). Nuclear localization of NF-κB (blue arrow) in a patient without H.E.L.P. therapy.
(h). Red arrow denotes nuclei staining by DAPI.

(i-k): Immunocytochemical localization of eNOS in placental villi (black arrows).
(i). Normal pregnancy.
(j). Patient on H.E.L.P. therapy.
(k).Patient without H.E.L.P. therapy.

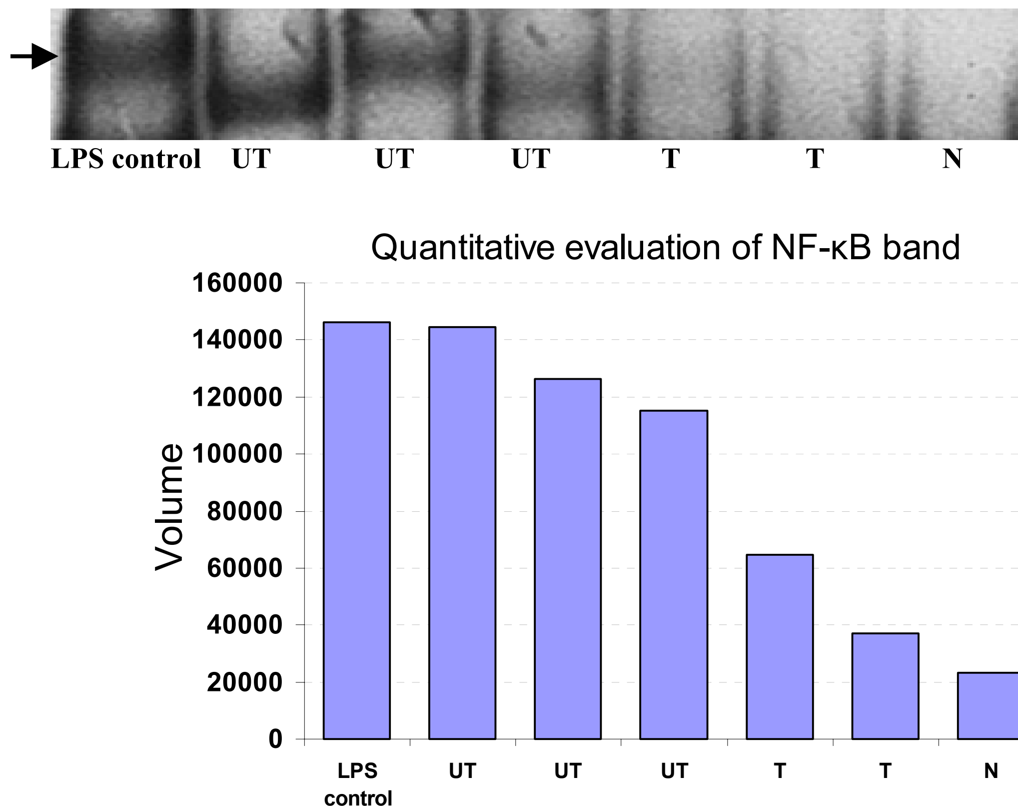
eNOS mainly exists in syncytiotrophoblast cells, but also stained positive in fetal endothelial cells in preeclamptic patient without H.E.L.P. therapy (double arrows).

3.4.7.2. EMSA analysis of NF-κB and AP-1 DNA binding activity:

Having established that in patients with preeclampsia undergoing H.E.L.P. therapy, NF-κB was mainly localized in cytoplasm in contrast to patients not undergoing this therapy where it was mainly loacalized in nuclei, it was necessary to establish the activation of NF-κB as well as AP-1 by gel mobility shift assay (EMSA). Intensity of NF-κB (P65/P50) was stronger in placental tissue from patients not undergoing H.E.L.P. therapy (Figure 7a). Quantification of the data by densitometry showed the H.E.L.P. reduced the activation of NF-κB. However no significant differences in activation of AP-1 were noted between the two groups (Figure 7b).

Figure 7a.

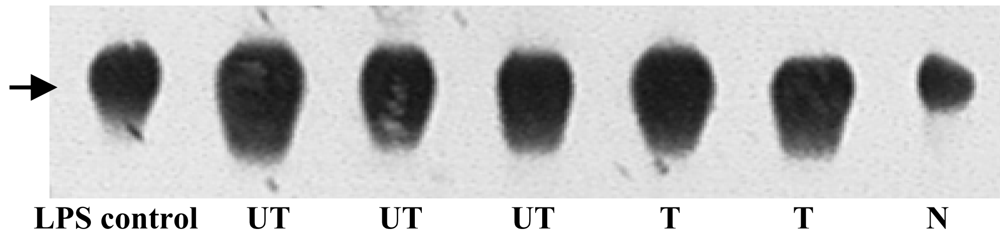
EMSA : NF-κB DNA binding activity (phosphorimager) from nuclear extracts of placental tissue.

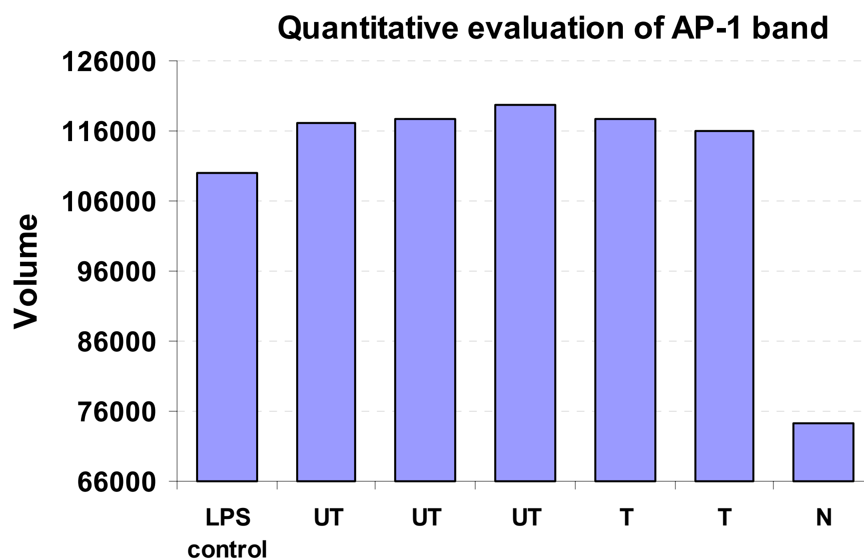


Detection of NF-κB activity by electrophoretic mobility shift assay (EMSA) from nuclear extracts of placental tissue. Control: placental tissue from normal pregnancy + LPS 10 μg/ml incubated for 1 hour at 37°C. T: placental tissue from preeclamptic patient under H.E.L.P.- apheresis. UT: placental tissue from preeclamptic patient without H.E.L.P.-apheresis. N: placental tissue from normal pregnancy. Arrow indicates P65/P50 heterodimer which was confirmed by supershift assay using antibodies to P65 and P50.

Figure 7b.

EMSA : AP-1 DNA binding activity (phosphorimager) from nuclear extracts of placental tissue.



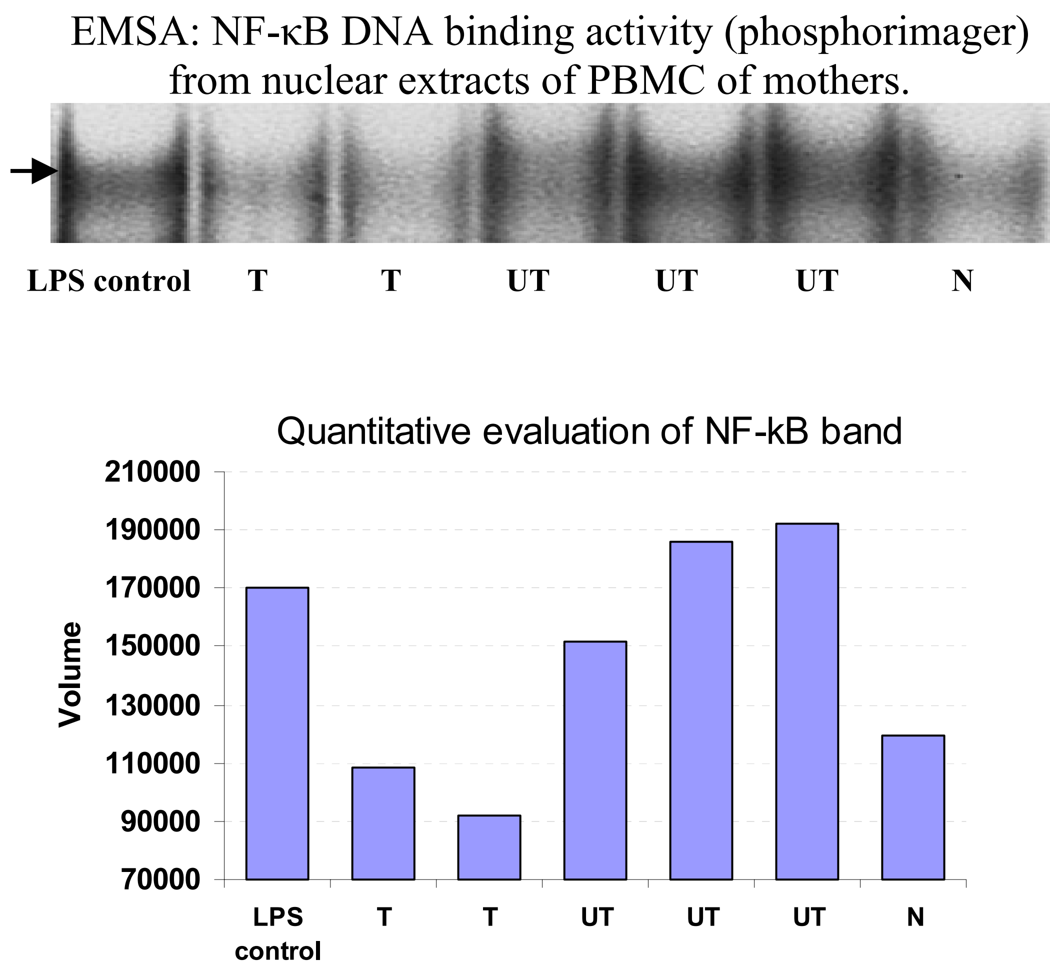


Detection of AP-1 activity by electrophoretic mobility shift assay (EMSA) from nuclear extracts from placental tissue. Control: placental tissue from normal pregnancy + LPS 10µg/ml incubated for 1 hour at 37°C.
T: placental tissue from preeclamptic patient under H.E.L.P.-apheresis. UT: placental tissue from preeclamptic patient without H.E.L.P.-apheresis. N: placental tissue from normal pregnancy. Arrow indicates AP-1 band.

3.4.8. Modulation of proinflammatory status in maternal PBMC:

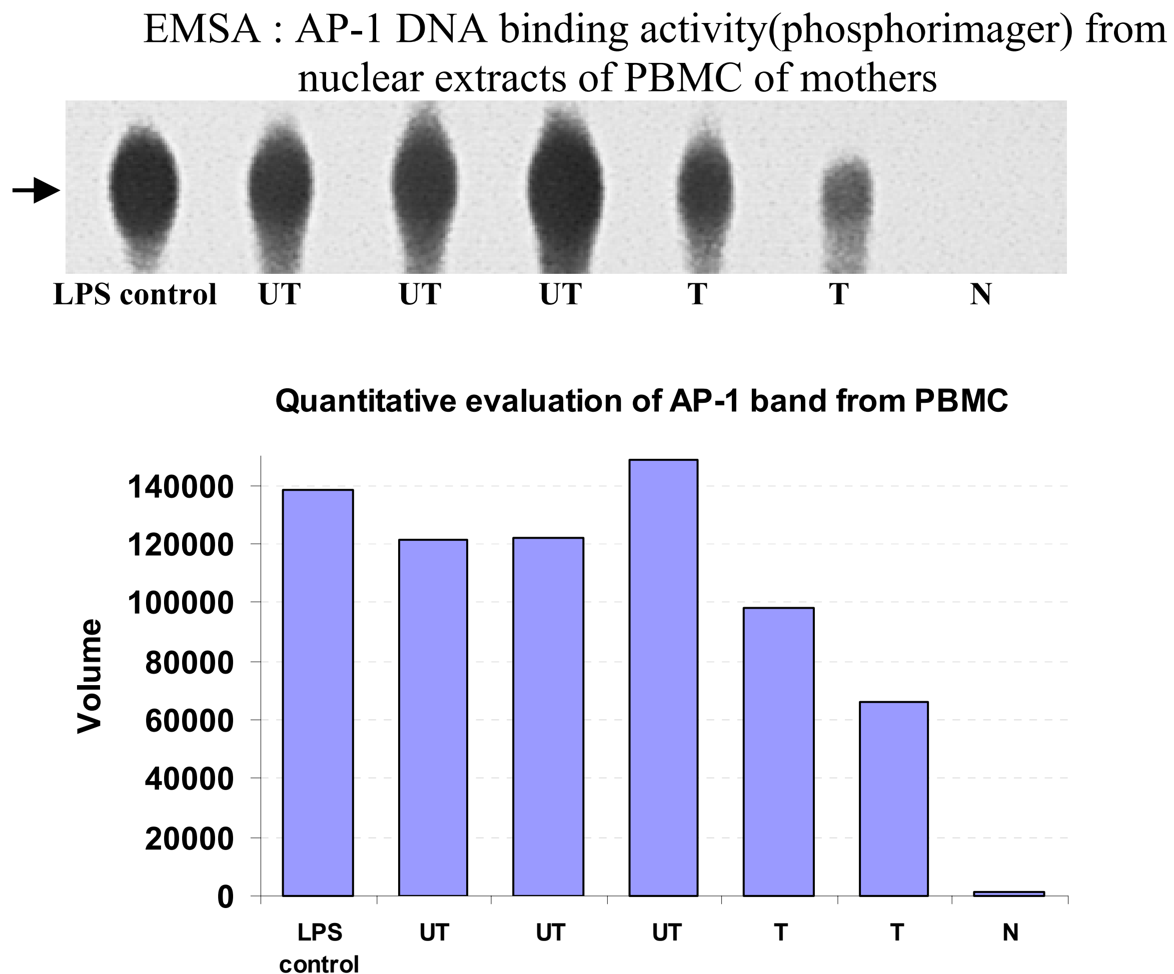
Activation of NF-κB and AP-1 was also measured in PBMC of preeclamptic patients treated with or without H.E.L.P. therapy. The data is shown in Figure 8 (a, b).

Figure 8a.



Detection of NF-κB activity by electrophoretic mobility shift assay (EMSA) from nuclear extracts of PBMC of mothers. Control: THP-1 + LPS 1 μg/ml incubated for 1 hour at 37°C. T: PBMC from preeclamptic patient under H.E.L.P.-apheresis. UT: PBMC from preeclamptic patient without H.E.L.P.-apheresis. N: PBMC from normal pregnancy. Arrow indicates P65/P50 heterodimer which was confirmed by supershift assay using antibodies to P65 and P50.

Figure 8b.



Detection of AP-1 activity by electrophoretic mobility shift assay (EMSA) from nuclear extracts from PBMC of mothers. Control: THP-1 + LPS 1µg/ml incubated for 1 hour at 37°C. T: PBMC from preeclamptic patient under H.E.L.P.-apheresis. UT: PBMC from preeclamptic patient without H.E.L.P.-apheresis. N: PBMC from normal pregnancy. Arrow indicates AP-1 band.

Patients undergoing H.E.L.P. therapy showed lower activation of NF-κB in PBMC as compared to patients not undergoing the apheresis. In contrast to placental tissue, similar patterns of activation were seen in NF-κB and AP-1.

4. Discussion

Preeclampsia is a multifactorial disorder of pregnancy. This may explain why till today its etiology and pathogenesis remain unknown. It is currently accepted that shallow endovascular cytotrophoblast invasion in the spiral arteries and endothelial dysfunction are two key features of preeclampsia resulting in dysregulated uteroplacental circulation and vascular tone (Antoinette et al., 2001).
Placental ischemia due to deficient spiral arterial blood supply to the placenta probably initiates endothelial disturbances both in the fetus and the mother (Chua et al., 1991; Smarason et al., 1993; Sargent et al., 1994). However it has been reported that endothelial dysfunction is already evident in the first trimester (Lockwood and Peters, 1990; Taylor et al., 1990). Whether a localized reduction in uteroplacental perfusion could lead to generalized vascular changes in the maternal circulation is still unclear.

Alterations in lipoprotein profile and metabolism have been suggested to play an important role in pathogenesis of preeclampsia. Brosens (1972) described it as “acute atherosis” of uteroplacental beds. Lp(a) was detected in the biopsy specimen of placental bed spiral arteries with atherosclerotic lesions (Meekins et al., 1994). It has been reported recently that circulating levels of Lp(a) are markedly increased in preeclamptic patients compared to normal pregnant women, and correlated with the severity of the disorder (Wang et al., 1998). Lp(a), by competing with plasminogen for its binding sites on fibrin clots and on endothelial cells, inhibits its activation to plasmin, which results in reduction of fibrinolysis (Hajjar et al., 1989). Recently it was reported that Lp(a) binds and inactivates tissue factor pathway inhibitor leading to or favoring thrombotic processes (Caplice et al., 2001). Thus, elevated circulating levels of Lp(a) may be involved in the atherothrombogenic process of preeclampsia. However it is still controversial whether high levels of Lp(a) correlate with severity of preeclampsia since its highly skewed distribution in individuals (as observed in our 6 preeclamptic patients and controls), as well as its progressive and highly individualized increments between each trimester did not correlate with changes in other lipoproteins (Belo et al., 2002).

Increased LDL and VLDL levels are characteristics of normal pregnancy and are elevated further in preeclampsia (Potter and Nestel, 1979). High levels of plasma triglycerides alter the diameter of LDL particles in women with preeclampsia (Satter et al., 1997). These small, dense LDL particles have an increased susceptibility to oxidative modification. Increased levels of antibodies to oxidized LDL have been reported in preeclamptic patients (Branch et al., 1994). In this present study, no significant differences in the circulating levels of triglycerides, VLDL, LDL and FFA between preeclamptic patients and normal pregnant women were observed. This discrepancy between previous studies and data reported here maybe because patients in this study were between second and third trimester while overt elevations in circulating lipids and particularly triglycerides have been reported to occur in the last trimester of pregnancy (Potter and Nestel, 1979; Sattar et al., 2000).
Alterations in the concentrations as well as lipid protein composition favor oxidative modification of lipoproteins. Oxidized LDL (oxLDL) induces changes in endothelial cell function by stimulating the expression of cell adhesion molecules. In vitro studies showed that expression of adhesion molecules are cytotoxic to vascular endothelial cells (Tatzber and Esterbauer, 1995). In addition they increased capillary permeability, platelet thrombosis and vascular tone (Roberts, 1993).

Endothelial dysfunction plays a major role in pathogenesis of preeclampsia and since dyslipidemia is an important factor in endothelial damage, this may explain the role of lipids in preeclampsia.

Recent studies strongly suggest that preeclampsia is not simply an imbalance between prostacyclin (PGI_2)/thromboxane (TXA_2) system, but other vasodilative and vasoconstrictive mediators such as nitric oxide (NO) and endothelin (ET-1) are also actively involved in pathogenesis of preeclampsia (Morris, 1996). However, the exact mechanism of action of NO and ET-1 in pathogenesis of preeclampsia still remains unresolved.
NO is synthesized from the amino acid L-arginine by nitric oxide synthetase (NOS). Three isoforms of NOS have been identified:

endothelial (eNOS), neuronal (nNOS) and inducible (iNOS) type. The eNOS is localized in vascular endothelium, platelets and the cardiac tissue. Endothelial cell NOS is activated by shear stress. NO is increased in normal pregnancy and is believed to be a major contributor to the physiological vasodilation of uncomplicated pregnancy (Myatt et al., 1992; Williams, 1997). Whether NO production or its secretion is reduced in human preeclampsia and also in animal models of pregnancy-induced hypertension is not clearly established (Khalil et al., 2002).
Maternal plasma levels of ET-1 are increased in preeclampsia when compared with normal pregnancy and the highest levels are found in HELLP syndrome (Schiff et al., 1992). ET-1 is the most potent vasoconstrictor and it is stimuli for the expression of cytokines and growth factors such as TNF, TGF-β, and PDGF in animal models (Nakamura et al., 1995). It also increases the expression of several genes including collagenase and transcription factors (Simonson, 1993). Thus ET-1 plays an important role in placental trophoblast implantation, platelet activation, intravascular coagulation and inflammatory processes.

Preeclampsia and inflammation share some common features. Increased circulating levels of proinflammatory cytokines such as IL-1, TNF-α and its soluble receptors (p55, p75) have been reported in preeclamptic patients (Vince et al., 1995). Primary proinflammatory cytokines including IL-1and TNF-α stimulate the production of chemokines (IL-8, MCP-1), as well as secondary proinflammatory cytokine IL-6 (Saadeddin et al., 2002). IL-6 stimulates endothelial cells and also provokes acute phase response such as hypoalbuminemia (Greer et al., 1994), increased production of acute phase proteins fibrinogen, LBP, and CRP by the liver (Castell et al., 1989).
Adhesion molecules (AMs) are induced on the endothelial surface and other vascular cells in response to various stimuli e.g. primary proinflammatory cytokines (IL-1, TNF-α). Their soluble forms arise from proteolytic cleavage of membrane bound molecules. Members of selectin family of AMs including lymphocyte L-selectin, E-selectin, P-selectin mediate sticking and rolling adhesion of leukocytes, whereas ICAM-1 and VCAM-1 mediate leukocyte arrest and migration

between the endothelial cells into the vessel wall (Jang et al., 1994). Preeclampsia is associated with increased maternal serum levels of soluble VCAM-1, E-selectin, and ICAM-1 (Toohey et al., 1994; Lyall et al., 1994).

Preeclampsia is characterized by maternal hypercoagulatory situation. Fibrinogen is a key coagulatory factor and acute phase reactant and is exclusively synthesized by the liver. It plays a central role in thrombosis and strongly modulates hemostasis, serum viscosity and platelet aggregation (Ernst et al., 1986). C-reactive protein (CRP), also an acute phase reactant, is actively involved in atherosclerotic processes. The possible mechanism of its direct involvement is complement activation and induction of synthesis of tissue factor by monocytes (Cermak et al., 1993). Tissue factor is a 47-kDa transmembrane protein and it initiates the extrinsic coagulatory pathway (Edginton et al., 1991). It is a proinflammmatory factor because its promoter region shares consensus sequences with NF-κB and AP-1 (Cui et al., 1994). Increased TF expression has been reported in preeclamptic placenta but not in placentas from normal pregnancies (Dechend et al., 2000).

Platelet activation is also a common feature in preeclampsia. Activated platelets release soluble inflammatory mediators such as CD40 ligand (CD40L) (Mellembakken, 2001), which is a member of TNF superfamily and is also expressed by variety of cells including activated T cells, monocytes, dendritic cells, endothelial cells, vascular smooth muscle cells, and epithelial cells (Mach et al., 1997). It combines with its receptor-CD40, which is a member of TNF receptor family and acts as central mediator during inflammatory processes. In vitro studies show that it is a potent monocyte stimulator inducing production of tissue factor and monocyte chemoattractant protein-1 (MCP-1) (Malik et al., 1996; Mach et al., 1997; Kuroima et al., 1999).

CD40-CD40L, an atherogenic mediator, is upregualted in patients with hypercholestrolemia (Semb et al., 2003), unstable angina (Aukrust et al., 1999) and diabetes (Marx et al., 2003). Human atheroma also expresses CD40-CD40L, which plays a potential role in processes involved in plaque rupture (Aukrust et al., 1999). It induces

matrix metalloproteinases (MMPs) such as interstitial collagenase (MMP-1), stromelysin (MMP-3) as well as TF expression and activity (Schonbeck et al., 1997). Interruption of CD40-CD40L provides a mechanism for atheroma stabilization. Since atheroma and preeclampsia share certain common features, inhibition of CD40-CD40L dyad and reduction of circulating soluble CD40L may prove beneficial in these patients (Kato et al., 2001).

Conventional therapeutic approaches for the management of preeclampsia have proved unsatisfactory. A plausible therapy which simultaneously reduces atherogenic lipoproteins (LDL and Lp(a)), procoagulatory-thrombogenic factors (fibrinogen, TF, and CD40L), proinflammatory markers (cytokines, AMs) and activation of transcription factors (NF-κB and AP-1) should prove beneficial in preeclampsia.

H.E.L.P. therapy fulfills most of these criteria. It effectively reduces LDL, Lp(a), fibrinogen and inflammatory parameters such as CRP and AMs (sICAM-1 and sVCAM-1) (Wieland et al., 2002; Klaus et al., 2002).

In this study we showed that single H.E.L.P. therapy reduced circulating levels of various proinflammatory cytokines (TNF-α, soluble TNF receptor p55 and p75), chemokines (MCP-1), LBP, AMs (sVCAM-1 and sE-selectin), procoagulatory factors (TF, CD40L, and homocysteine) and vasoconstrictor ET-1.

Three preeclamptic patients treated with H.E.L.P.-apheresis differed in their lipid, proinflammatory and procoagulatory status. Even though H.E.L.P. procedure was similar in all patients, yet the frequency and number of treatments need individual approach.
Basically, the progressive dyslipidemia in all the three patients were interrupted. Repeated therapies at short intervals maintained their plasma lipoprotein and fibrinogen levels lower than their baseline values. This was significant in patient Nr.3 who had high circulating levels of Lp(a) and fibrinogen (87 and 609 mg/dl respectively). Concomitant with the reduction of fibrinogen, plasma viscosity decreased, uteroplacental circulation shear stress decreased resulting

in improved placental perfusion rate. Immunohistochemical studies showed that eNOS staining was intense in preeclamptic patients without H.E.L.P. therapy compared to those undergoing H.E.L.P. therapy. Patients without H.E.L.P. therapy demonstrated positive staining for fetal endothelium. Taken together data from the present study showed that H.E.L.P. therapy reduced maternal circulating ET-1, reduced the eNOS expression and possibly improved endothelial function and placental ischemia.

As described already, preeclampsia involves endothelial dysfunction, maternal inflammatory responses, complement system activation, leukocyte stimulation, and thrombosis. Transcription factor NF-κB plays a central role in all these processes. However, its role in preeclampsia still remains unknown. It plays a pivotal role in systemic inflammatory responses and should be considered a target for therapy. Proinflammatory markers including TNF, colony stimulating factors (G-CSF and GM-CSF), interleukins (IL-1β, IL-2, IL-6, IL-12), interferons (IFN-β), chemokines (IL-8, Rantes, MCP-1), AMs (ICAM-1, E-Selectin, VCAM-1), and enzymes (COX-2, iNOS) are regulated by NF-κB (Christman et al., 1998).
Data from the present study are in accord with those reported recently by Dechend (2003) who showed NF-κB was activated in placenta from preeclamptic patients. Preeclamptic patients undergoing repeated H.E.L.P. therapy demonstrated reduced activation of NF-κB compared to patients without H.E.L.P. therapy both by EMSA technique and immunohistochemical studies. Translocation of NF-κB from cytoplasma to nucleus was more evident in patients without H.E.L.P. therapy than those undergoing H.E.L.P. treatment. AP-1 is a family of related transcription factors which usually consists of c-Jun/c-Fos heterodimer or c-Jun/c-Fos homodimer. Changes in AP-1 activity in response to extracellular signals is regualted both on the levels of transcription of Jun and Fos genes and by posttranscriptional modification of preexisting AP-1. It is activated in various pathological situation (Penner et al., 2001). However activation of NF-κB and AP-1 are differently regulated (Tran-Thi et al., 1995; Penner et al., 2001; Roebuck et al., 1999). This might explain differences in activation of these transcription factors in placental tissue and maternal PBMC observed in this study.

However, we must mention that the preeclamptic patients involed in this study in treated and not-treated groups are not fully matched clinically. A more randomized study with larger number of patients is needed to prove the beneficial effect of H.E.L.P. therapy in preeclampsia.

Summary

Preeclampsia, a multisystem disorder of pregnancy is associated with significant morbidity and mortality both in mother and baby. Worldwide an estimated 600,000 women die each year of pregnancy-related causes, and 99% of these deaths occcur in developing countries (Magpie Trial, 2002). It has been reported that preeclampsia or eclampsia account for more than 50,000 maternal deaths per year (Duley, 1992).

Preeclampsia results from initiatial placental trigger and failure of normal invasion of trophoblast cells, leading to maladaption of maternal spiral arterioles (Meekins et al., 1994). Maternal arterioles are source of blood supply to fetus, and maladaption of these vessels can interfere with normal villous development. This may then lead to secondary damages such as fibrin deposition, thrombotic and coagulatory disturbances and lipid deposition in placenta. Persistent placental underperfusion stimulates release of toxic factors which come in contact with maternal circulation resulting in vascular dysfunction. Decreased endothelial prostacyclin synthesis, reduced bioavailability of nitric oxide, greater cell permeability and increased expression of endothelial cell adhesion molecules and prothrombotic factors contribute to maternal disorders (Dekker and Sibai, 1998). All these changes stress the importance of maternal endothelium during preeclampsia. Hyperlipoproteinemia as well as proinflammtory markers are deleterious to endothelium. So far no therapeutic intervention proved satisfactory to prevent or delay the onset of this disease except cesarean section (Sibai, 1998).

A therapeutic approach which would correct dyslipoproteinemia and reduce the circulatory levels of fibrinogen, proinflammatory markers, and improve the procoagulatory and prothrombotic situation may prove beneficial in preeclamptic patients.
Heparin-mediated extracorporeal low-density lipoprotein precipitation drastically reduces LDL, Lp(a), and fibrinogen. It improves procoagulatory and prothrombotic situation in CHD patients. Since most of these factors are present in preeclampsia, H.E.L.P. apheresis

was initiated in these patients. Results and outcome of this study are summarized as follows:

1. No differences in circulating levels of lipoproteins or their profile were evident in preeclamptic patients and normal pregnant women who delivered by cesarean section. The lipid-protein composition and apolipoprotein profile of lipoproteins isolated from plasma by sequential density gradient centrifugation did not show remarkable differences.

2. In a pilot study on CHD patients, H.E.L.P. therapy reduced circulating levels of proinflammatory markers including adhesion molecules (sVCAM-1, sE-selectin), MCP-1, ET-1, LBP, hs-CRP and prothrombotic factors such as tissue factor and homocysteine.

3. On the basis of these data, we treated three from six preeclamptic patients with the apheresis system. Patients toletated H.E.L.P.-apheresis well and without any untoward adverse events.

4. With repeated H.E.L.P. therapies, circulating levels of LDL, Lp(a), fibrinogen and proinflammatory markers were maintained at low levels. Gestational age in these women was prolonged, babies delivered by cesarean section showed normal physiological functions and development during their neonatal stage. Both the mothers and babies who are checked at regular intervals are clinically well.

5. Newborns from three preeclamptic patients who did not undergo H.E.L.P. therapy, one died immediately after birth, other two suffered from severe perinatal complications. But all three mothers recovered quickly after cesarean section.

6. H.E.L.P. therapy also corrected the proinflammtory situation in placenta and maternal circulation as reflected by reduced activation of NF-κB in placental tissue and maternal PBMC compared to data from patients not undergoing this therapy.

In conclusion, data from the present study though on small number of patients demonstrated that H.E.L.P.-apheresis may prove a beneficial therapy in the management of preeclamptic patients. It deserves consideration in designing future therapeutic concepts for treating preeclampsia.

Zusammenfassung

Präeklampsie, eine Multisystemerkrankung in der Schwangerschaft, ist mit einer signifikant erhöhten Morbidität und Mortalität assoziiert. Pro Jahr sterben weltweit ca. 600 000 Frauen an schwangerschafts-assoziierten Komplikationen, 99% davon in den Entwicklungsländern (Magpie trial, 2002). An einer Präeklampsie oder Eklampsie selbst sterben mehr als 50 000 Frauen pro Jahr (Duley, 1992).

Pathogenetisch resultiert die Präeklampsia aus initialen plazentaren Triggern und einer fehlerhaften Invasion von Trophoblastenzellen. Dies wiederum führt zur mangelhaften Adaption maternaler Spiralarteriolen (Meekin et al., 1994). Maternale Arteriolen sichern physiologischerweise die Blutversorgung des Feten und eine Maladaption dieser Gefäße kann die normale villöse Entwicklung beeinträchtigen. Dies führt zu Fibrinablagerungen, thrombotischen und koagulatorischen Störungen sowie zu Fettablagerungen in der Plazenta. Die hierdurch persistierende Minderdurchblutung der Plazenta bewirkt eine Freisetzung von toxischen Mediatoren, die im maternalen Kreislauf eine vaskuläre Dysfunktion induzieren. Weiterhin tragen eine verminderte endotheliale Prostacyclinsynthese, eine verminderte Bioverfügbarkeit von Stickoxid, eine gesteigerte Zellpermeabilität und eine gesteigerte Expression endothelialer Adhäsionsmoleküle und prothrombotischer Faktoren zum mütterlichen Krankheitsbild bei (Dekker et al., 1998). Diese Befunde weisen auf die Bedeutung des mütterlichen Gefäßendothels bei der Präeklampsie hin. Insbesondere die Hyperlipoproteinämie und die Freisetzung proinflammatorischer Mediatoren schädigen das Endothel. Bis heute konnte mit keiner therapeutischen Intervention eine Verbesserung der Erkrankung oder ein Hinauszögern der Schwangerschaft erreicht werden. Aus diesem Grund wird bei den Patientinnen bisher eine elektive Sektio durchgeführt.

Die Pathogenese der Erkrankung weist darauf hin, dass die Verminderung der zirkulierenden Spiegel von Fibrinogen und proinflammatorischen Mediatoren im mütterlichen Kreislauf sowie die Verbesserung der prokoagulatorischen und prothrombotischen Situation in der Therapie der Präeklampsie erfolgreich sein könnten.

Die Heparininduzierte Extrakorporale LDL Präzipitation (H.E.L.P.) führt zu einer drastischen Reduktion von LDL, Lp(a) und Fibrinogen. Darüber hinaus verbessert diese Therapie den prokoagulatorischen und prothrombotischen Zustand bei KHK-Patienten.

Da all diese Faktoren auch bei der Präeklampsie eine entscheidende Rolle spielen, wurde in der vorliegenden Studie der Einsatz der H.E.L.P.-Therapie bei diesen Patientinnen untersucht. Die Ergebnisse der Studie lassen sich wie folgt zusammenfassen:

1. Im Vergleich zu gesunden Schwangeren die mit Sektion entbunden wurden, konnten bei Patientinnen mit Präeklampsie keine Unterschiede in der Lipoproteinkonzentration und im Lipoproteinprofil festgestellt werden. Der Lipid-Protein- und Apolipoproteingehalt, der aus dem Plasma präeklamptischer Patientinnen mittels sequentieller Ultrazentrifugation isolierten Lipoproteine, wies keine abnorme Zusammensetzung auf.

2. Durch die H.E.L.P.-Therapie konnten in einer Pilotsstudie mit KHK-Patienten sowohl die Plasmakonzentration proinflammatorischer Marker wie VCAM-1, E-Selektin, MCP-1, ET-1, LBP, hs-CRP als auch die Konzentrationen prothrombotischer Faktoren wie Tissue Factor und Homocystein signigikant reduziert werden.

3. Auf Grund dieser Befunde wurden 3 von insgesamt 6 Patientinnen mit Präeklampsie mittels der H.E.L.P.-Therapie behandelt. Alle drei Patientinnen tolerierten die Behandlung gut und zeigten keinerlei Nebenwirkungen.

4. Durch die wiederholten H.E.L.P.-Therapien konnten bei diesen Patientinnen die LDL-, Lp(a)- und Fibrinogenspiegel dauerhaft abgesenkt werden. Gleichzeitig wurde das Gestationsalter verlängert, die entbundenen Babies wiesen in der neonatalen Phase normale physiologische Funktionen und einen normalen Entwicklungsstand auf. Die Mütter und Kinder wurden anschließend regelmäßig nachuntersucht und zeigten keinerlei klinische Auffälligkeiten.

5. Von den Neugeborenen der drei Präeklampsie-Patientinnen, die nicht mit der H.E.L.P.-Therapie behandelt wurden, verstarb eines, die beiden anderen entwickelten schwere perinatale Komplikationen. Die drei Mütter dieser Kinder erholten sich jedoch schnell.

6. Der durch die H.E.L.P.-Therapie korrigierte proinflammatorische Zustand der Plazenta wird durch eine, im Vergleich zum Plazentagewebe der drei unbehandelten Patientinnen, verminderte Aktivierung von NF-kappaB reflektiert. Durch die Therapie wird der Kernbindungsfaktor NF-kappaB, welcher für das Entzündungsgeschehen von zentraler Bedeutung ist, sowohl im plazentaren Gewebe als auch im maternalen Kreislauf – in den PBMC – in seiner Aktivität reduziert.

Obwohl bisher erst wenige Patientinnen behandelt wurden, zeigen diese Daten, dass die H.E.L.P.-Therapie eine vielversprechende Therapieoption in der Behandlung der Präeklampsie darstellen könnte. Bei weiteren Studien zur Behandlung der Präeklampsie sollte diese Behandlungsoption berücksichtigt werden.

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Glossary

ACAT	Acetyl CoA-cholesterol acyl transferase
ACE	3-Amino-9-ethyl-carbazole
AMs	Adhesion molecules
ApoA	Apolipoprotein A
ApoB	Apolipoprotein B
ApoC	Apolipoprotein C
ApoE	Apolipoprotein E
BP	Blood pressure
AP-1	Activator protein-1
BSA	Bovine serum albumin
bZIP	Leucine zipper
CETP	Cholesterol ester transfer protein
CHD	Coronary heart disease
COX-2	Cyclooxygenase-2
CS	Cesarean section
CSF	Colony stimulating factor
CTG	Cardiotocography
DAPI	4´,6-Diamidino-2-phenylindole
dATP	Deoxyadenosinetriphosphate
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EASIA	Enzyme amplified sensitivity immunoassay
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
EMSA	Electrophoretic mobility shift assay
eNOS	Endothelial nitric oxide synthetase
E-selectin	Endothelial selectin
ET-1	Endothelin-1
FFA	Nonesterified fatty acid; Free fatty acid
FITC	Fluorescein isothiocynate
FPIA	Fluorescence polarization immunoassay
HCT	Hematocrit
Hcy	Homocysteine
HDL	High density lipoprotein
HDL-C	HDL-cholesterol
HELLP	Hemolysis, Elevated Liver enyzmes and Low Platelet count Syndrome
H.E.L.P.	Heparin-mediated extracorporeal low-density lipoprotein precipitation

HEPES	N-(2-Hydroxyethyl)piperazine-N´-(2-ethansulfonic acid)
HMG CoA	3-Hydroxy-3-methylglutaryl CoA
HRP	Horse reddish peroxidase
hs-CRP	High sensitive C-reactive protein
ICAM-1	Intercellular adhesion molecule-1
IDL	Intermediate density lipoprotein
IFN-β	Interferon-beta
IL-1	Interleukin-1
iNOS	Inducible nitric oxide synthetase
IUGR	Intrauterine growth retardation
LBP	Lipopolysaccharide binding protein
LCAT	Lecithin:cholesterol acyltransferase
LDL	Low density lipoprotein
LDL-C	LDL-cholesterol
Lp(a)	Lipoprotein (a)
LPS	Lipopolysaccharide
MCA	Middle cerebral artery
MCP-1	Monocyte chemoattractant protein-1
NF-κB	Nuclear factor-kappa B
NO	Nitric oxide
nNOS	Neuronal nitric oxide synthetase
oxLDL	Oxidized low-density lipoprotein
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PDGF	Platelet derived growth factor
PGI_2	Prostaglandin I_2
PMSF	Phenylmethylsulfonylfluoride
RNA	Ribonucleic acid
sCD40L	Soluble CD40 ligand
SMCs	Smooth muscle cells
TF	Tissue factor
TGF	Transforming growth factor
TNF	Tumor necrosis factor
TNF r55	Tumor necrosis factor receptor p55
Tris	Tris-(hydroxymethyl)-aminomethane
TXA_2	Thromboxane A_2
UA	Umbilical artery
VCAM-1	Vascular cellular adhesion molecule-1
VLDL	Very low density lipoprotein
VLDL-C	VLDL-cholesterol

Curriculum Vitae

Family name	Wang
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Primary and high school	
1978-1983	Primary school in Yantai, Shandong, China
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Medical education	
1989-1994	Studied in Medical College of Shandong University, P.R.China. Internship in Shandong Provincial Hospital, P.R.China. Obtained Bachelor´s Degree in Medicine (M.B.).
1994-1997	Studied on Pediatric Medicine in Medical College of Beijing University. Resident in Pediatric Department of Third Affiliated Hospital of Medical College of Beijing University (Head of department Prof. Dr. Hongmao Ye). Obtained Master´s Degree in Pediatric Medicine (under the guidance of Prof. Dr. Meizhu Li).
1997-2000	Pediatrician in Children´s Hospital of Guangzhou, Canton, P.R.China.
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