Available online at www.sciencedirect.com
ScienceDirect
Transfusion and Apheresis Science 35 (2006) 103–110
TRANSFUSION AND APHERESIS SCIENCE
intl.elsevierhealth.com/journals/tras

Heparin-mediated extracorporeal low density lipoprotein precipitation as a possible therapeutic approach in preeclampsia

Ying Wang [a,*], Autar K. Walli [a], Andreas Schulze [b], Frithjof Blessing [a], Peter Fraunberger [a], Christian Thaler [c], Dietrich Seidel [a], Uwe Hasbargen [c]

[a] *Institute of Clinical Chemistry, University Hospital Grosshadern, Marchioninistrasse 15, 81377 Munich, Germany*
[b] *Division of Neonatology, Klinikum Grosshadern, University of Munich, Germany*
[c] *Division of Obstetrics and Gynaecology, Klinikum Grosshadern, University of Munich, Germany*

Received 3 November 2005; received in revised form 29 April 2006; accepted 18 May 2006

Abstract

Preeclampsia is a pregnancy-related hypertensive disease resulting in substantial maternal and neonatal morbidity and mortality. Until today there is no satisfactory treatment to stop disease progression except immediate delivery of the fetus. Heparin-mediated extracorporeal low density lipoprotein (LDL) precipitation (H.E.L.P.) apheresis removes simultaneously circulating LDL, lipoprotein(a) [Lp(a)], fibrinogen, C-reactive protein (CRP) and various proinflammatory and procoagulatory factors. This study was to test the feasibility of H.E.L.P. apheresis in preeclamptic patients and its potential effects on blood and placental markers of preeclampsia. We applied H.E.L.P. apheresis to nine preeclamptic patients and it was well tolerated. Their gestational ages could be continued by 17.7 (3–49) more days. Eight of the nine neonates did well during their neonatal stage. One infant died of late-onset sepsis. H.E.L.P. apheresis reduced significantly circulating levels of triglycerides, total and LDL-cholesterol, Lp(a), fibrinogen, hs-CRP, TNFα, sVCAM-1, E-selectin, lipopolysaccharide binding protein (LBP), homocysteine and plasma viscosity. We conclude that H.E.L.P. apheresis reduced maternal circulating levels of proinflammatory and coagulatory markers and plasma viscosity without overt maternal or neonatal clinical side effects.

Keywords: Preeclampsia; Heparin-mediated extracorporeal low density lipoprotein precipitation (H.E.L.P.) apheresis; Endothelial dysfunction; Inflammation; Coagulation

1. Introduction

Preeclampsia which affects about 6–8% of pregnancies is a major cause of maternal and neonatal morbidity and mortality [1]. It is a multisystem disorder characterized by hypertension, proteinuria, oligouria, pulmonary edema, impaired liver function, thrombocytopenia and disseminated intravascular coagulation. Risks to the fetus include growth retardation, placental abruption and death. HELLP syndrome, a potential life-threatening complication occurs in 0.17–0.85% of all live births leading to hemolysis (H), elevated liver enzymes (EL) and low platelet count (LP) [2].

* Corresponding author. Tel.: +49 89 7095 3239; fax: +49 7731 181112.
E-mail address: cathy.wy@lrz.uni-muenchen.de (Y. Wang).

1473-0502/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.transci.2006.05.010

The cause of preeclampsia remains unclear. The current understanding of preeclampsia suggests that: (i) insufficient uteroplacental circulation either because of deficient placentation or acute atherosis in vasculature of the placental bed, (ii) secondary systemic maternal endothelial dysfunction and (iii) ill-defined links between the two [3]. Multiple factors have been associated with maternal endothelial dysfunction including alterations in lipoprotein concentrations and lipid–protein composition [4,5], proinflammatory markers such as cytokines [6], chemokines, adhesion molecules [7] and procoagulatory markers such as fibrinogen, C-reactive protein (CRP), tissue factor (TF) [8], and homocysteine [9].

There is no treatment except for the immediate removal of the trophoblast, resulting in discontinuation of pregnancy. Prolongation of pregnancy is potentially advantageous for the fetus but deleterious for the mother. Any specific therapeutic approach need to be beneficial for both the mother and her fetus.

Heparin-mediated extracorporeal LDL precipitation (H.E.L.P.) apheresis effectively removes LDL, Lp(a), fibrinogen and CRP from the circulation and has been in clinical use since 1985. Heparin at low pH of 5.12 forms complexes not only with proatherogenic lipids but also with procoagulatory markers. The main indications for the apheresis are familial hypercholesterolemia, CHD and after heart transplantation [10]. We have recently shown that H.E.L.P. apheresis removes also circulating markers such as adhesion molecules, monocyte chemoattractant protein-1 (MCP-1), lipopolysaccharide binding protein (LBP), TF, soluble CD40 ligand (sCD40L), endothelin-1 (ET-1) and homocysteine [11].

We hypothesized that LDL-apheresis might also be potentially beneficial in women with preeclampsia. In this pilot study we addressed the following questions: whether H.E.L.P. apheresis was feasible in preeclamptic patients, whether it reduced circulating levels of proatherogenic lipoproteins, prothrombotic and proinflammatory markers and plasma viscosity in preeclamptic patients without overt side effects.

2. Materials and methods

2.1. Subjects

This study protocol was reviewed and approved by institutional review committee of University of Munich and all the patients included in this study gave their written informed consent. Thirteen preeclamptic patients, caucasian, 33.0 (23.9–39.8) years old, were enrolled. They fulfilled the diagnostic criteria of American College of Obstetricians and Gynecologists (1996): onset of hypertension during pregnancy (BP $\geqslant$ 140/90 mm Hg on two occasions $\geqslant$ 6 h apart after 20 weeks' gestation) with detectable urinary protein ($\geqslant$1 + by dipstick or $\geqslant$300 mg/24 h) and/or pathologic edema. Proteinuria was present in eight from these 13 preeclamptic patients at admission. Exclusion criteria were preexisting chronic hypertension, renal disease, diabetes, maternal sepsis and intrauterine infection. Thirteen age-matched women, caucasian, 33.9 (24.8–38.4) years old, normotensive, who had an uncomplicated course of pregnancy and delivered at term were also included in this study in order to obtain reference data in normal pregnancy. Clinical characteristics and laboratory data of all the patients are given in Table 1. All the preeclamptic patients at admission were receiving antihypertensive drugs, which were continued by using oral metoprolol or α-methyldopa after admission. Antenatal steroids were given immediately after admission (two doses of betamethasone 12 mg, 24 h apart, intramuscularly). Nine of the 13 preeclamptic patients were treated with H.E.L.P. apheresis. The remaining four preeclamptic patients, who either refused to undergo H.E.L.P. apheresis or experienced a rapid progression of preeclampsia were compared to those undergoing H.E.L.P. apheresis. All the patients enrolled underwent caesarean section. None of them was labouring before the section. Delivery in preeclamptic patients was initiated because of disease progression or an unsatisfactory fetal status which was confirmed by cardiotocography or Doppler ultrasound findings. All newborns were treated immediately by neonatologists and were transferred to a level III neonatal ICU. Exogenous surfactant treatment was required soon after the birth in all newborns.

2.2. Sampling

Venous blood samples were collected on day of admission in all patients, before (pre-) and after (post-) each apheresis additionally in preeclamptic patients undergoing H.E.L.P. apheresis. Placental venous blood was collected swiftly after the separation of the placenta from the uterus.

Table 1
Characteristics of patients enrolled and their neonates

	Mothers at admission		Neonates at birth	
	UP	PE	UP	PE
Number of patients, *n*	13	13	13	13
Gestational age, weeks	38 (35–40)	27 (24–32)*	–	–
BMI, kg/m^2	26 (23–37)	27 (20–46)	–	–
Primigravidas, *n*	8	10	–	–
Family history of PE, *n*	0	2	–	–
Birth weight, kg	–	–	3.4 (2.8–4.4)	0.9 (0.3–2.0)*
pH of UA blood	–	–	7.3 (7.2–7.3)	7.3 (7.1–7.4)
Lipid profile (mmol/L)				
Triglycerides	2.2 (1.1–3.0)	2.1 (0.7–3.6)	0.3 (0.2–0.4)	0.5 (0.3–0.7)*
Total cholesterol	5.5 (3.3–8.5)	6.5 (4.2–7.7)	1.7 (1.2–2.5)	2.3 (1.0–3.7)
LDL-cholesterol	3.4 (1.7–5.5)	3.7 (2.0–4.9)	0.7 (0.3–1.1)	1.2 (0.4–2.8)*
VLDL-cholesterol	0.7 (0.4–1.2)	0.7 (0.1–1.7)	0.2 (0.1–0.5)	0.3 (0.1–0.4)
HDL-cholesterol	1.6 (0.9–2.3)	1.8 (1.0–2.1)	0.8 (0.4–1.4)	0.5 (0.3–1.0)
Non-esterified fatty acids	1.0 (0.6–1.5)	1.5 (0.5–2.2)*	0.3 (0.2–0.7)	0.2 (0.2–0.3)
Lp(a) (mg/dL)	19 (0–74)	41 (10–214)	5 (0–11)	0 (0–9)
Angiogenic and antiangiogenic factors				
PlGF (pg/mL)	111 (72–294)	54 (15–143)*	–	–
VEGFR-1 (ng/mL)	1.8 (1.2–3.7)	4.8 (0.2–12.8)*	–	–

UP: uncomplicated pregnancy. PE: preeclampsia. UA: umbilical artery.
* Statistically significant difference ($P < 0.05$).

2.3. H.E.L.P. apheresis

The procedure for H.E.L.P. apheresis (Plasmat Futura®, B.Braun, Melsungen, Germany) has been described previously [12]. In brief, blood was drawn from a double lumen central venous catheter implanted via Seldinger technique. Blood cells were separated from plasma which was then mixed with acetate-heparin buffer pH 4.85 (ratio 1:1) to precipitate fibrinogen, LDL and Lp(a). The precipitate was then removed from the suspension by filtration. 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. Plasma free from LDL, fibrinogen and Lp(a) was mixed with cell-rich blood fraction and returned back to the patients. The average plasma volume treated was 3 L per session. Generally, plasma fibrinogen levels above 4 g/L, stable Doppler ultrasound findings and patient's condition were the criteria for performing a next apheresis session.

2.4. Plasma lipoproteins

VLDL/IDL, LDL, and HDL (solvent density 1.006–1.019, 1.019–1.063 and 1.063–1.21 g/mL, respectively) were isolated from plasma by sequential ultracentrifugation according to previously described methods [13].

2.5. Analytical techniques

Serum lipids was measured by routine automatic analyzer (Hitachi 911, Roche, Germany), in which Lp(a) was measured by method of immunoturbidimetry (Wako, Germany). Plasma non-esterified fatty acids (FFA) was measured by NEFA C kit (Wako, Germany). TNF r55 and TNF r75 were measured automatically on Cobas Core® immunological analyzer by enzyme-linked immunobinding assays (Hoffmann La Roche Ltd, Basel, Switzerland) with a detection limit of 100 pg/mL. IL-6 was measured by solid phase enzyme amplified sensitivity immunoassay (EASIA) (Biosource, Europe S.A.) on the Cobas Core® with a detection limit of 1.5 pg/mL. Fibrinogen was measured according to Clauss method (STA-R, Roche). Hs-CRP was measured by method of immunoturbidimetry (Cobas Integra, Roche, Germany) with a detection limit of 8.5 µg/dL.

Serum sVCAM-1, sICAM-1, sE-selectin, ET-1, PlGF, VEGFR1 were measured by ELISA kits (R&D, USA). Serum TNF-α was measured by method of EASIA (Biosource, Europe S.A.). Serum LBP was measured by chemiluminescence sandwich

immunoassay (Immulite®, USA). Plasma homocysteine was measured by fluorescence polarization immunoassay (Imx®, USA). TF was measured in sodium citrate plasma by ELISA kit (Loxo GmbH, Germany). Possible hemodilution for markers after each apheresis was corrected by hematocrit.

2.6. Statistical analysis

All the values are presented as median (range). Statistical significance was evaluated by student *t*-test or Wilcoxon's test using the SAS software (version 8.2, SAS Institute Inc, NC). For all analyses, $P < 0.05$ was considered significant.

3. Results

3.1. Circulating levels of lipids, proinflammatory and procoagulatory markers

No significant differences in lipids, lipoprotein profile, lipid–protein composition in isolated lipoproteins (data not shown) and homocysteine were noted between preeclamptic and uncomplicated pregnant women (average gestational ages 27 and 38 weeks, respectively). However, plasma non-esterified fatty acids (FFA) levels were higher in preeclamptic women ($P < 0.05$). Lp(a) levels were not significantly different between preeclamptic and normal pregnancy. This may due to the highly skewed character of Lp(a) and the small number of patients. However individualized high Lp(a) levels (up to 214 mg/dL, reference < 30 mg/dL) were observed (7 in 13 preeclamptic patients).

We observed lower levels of PlGF and higher levels of VEGFR-1 in preeclamptic patients compared to normal pregnant women after corrected by gestational age [14]. Compared to neonates born from uncomplicated pregnant mothers, neonates from preeclamptic mothers had elevated levels of serum triglycerides and LDL-cholesterol (Table 1).

TNF receptor p55 (TNF r55), hs-CRP, E-selectin and LBP were elevated in preeclamptic women compared to uncomplicated pregnant women. Fibrinogen and sVCAM-1 tended to be higher in preelclamptic women but these differences were statistically insignificant (Table 2).

3.2. Clinical outcomes of preeclamptic women and their neonates

In the 9 patients undergoing H.E.L.P. apheresis, their pregnancy could be continued on average for 17.7 more days (3–49 days) after the initiation of the apheresis. Eight of them experienced improvement in their blood pressure, proteinuria and edema. One patient developed HELLP syndrome within 3 days after first apheresis (patients Nr. 5 in Table 3) and immediate caesarean section was necessary. Among the four patients who had preeclampsia but were not treated with the apheresis, one progressed into eclampsia the same day after hospitalization, one was suspected placental abruption after placement of central venous catheter. Both of the two patients received an emergency caesarean section. The other two patients showed progressive pathological changes in Doppler ultrasound after hospitalization. All the 13 preeclamptic patients were clinically stable after delivery and recovered without complications. Antihypertensive medications were discontinued soon after discharge. No obvious differences were

Table 2
Circulating proinflammatory and procoagualtory parameters in uncomplicated pregnant and preeclamptic women

Parameters	Mothers at admission		
	Uncomplicated pregnancy	Preeclampsia	*P*
TNF-α (pg/mL)	30 (13–63)	29 (28–42)	NS
IL-6 (pg/mL)	5 (2.5–13.1)	5 (1.4–24.8)	NS
TNF r55 (ng/mL)	1.8 (1.4–4.9)	2.2 (1.2–4.8)	0.046
TNF r75 (ng/mL)	3.9 (2.6–5.3)	4.2 (2.0–7.2)	NS
LBP (μg/mL)	6.9 (4.1–11.3)	7.9 (5.7–10.1)	0.005
sVCAM-1 (ng/mL)	563 (449–867)	805 (414–1064)	NS
E-selectin (ng/mL)	25 (7–32)	43 (20–71)	0.028
Homocysteine (μmol/L)	5.1 (3.3–8.3)	5.5 (4.2–8.0)	NS
Fibrinogen (g/L)	4.2 (2.7–5.6)	4.4 (2.7–6.4)	NS
hs-CRP (mg/dL)	3 (0.5–6)	12 (3–51)	0.007

NS: no statistical significance ($P > 0.05$). LBP: lipopolysaccharide-binding protein.

Table 3
Clinical outcomes of 13 preeclamptic patients and their neonates

	Patients with H.E.L.P. apheresis									Patients without H.E.L.P. apheresis			
	1	2	3	4	5	6	7	8	9	10	11	12	13
Mothers													
BP at admission (S/D) (mm Hg)	156/108	140/90	140/80	130/60	130/80	130/90	150/90	150/90	145/95	150/110	145/90	130/85	210/145
Gestation age at admission (weeks)	$24^{+1\$}$	26^{+2}	27^{+3}	27^{+4}	23^{+6}	25^{+4}	28^{+4}	24	26^{+4}	31^{+6}	23^{+5}	28^{+6}	27^{+1}
Magnesium sulfate	+	–	–	+	+	+	+	+	+	+	+	+	+
H.E.L.P. apheresis													
Number of apheresis	3	6	4	7	1	1	1	2	6	–	–	–	–
Prolongation of gestation (days)	5	23	17	23	3	19	11	9	49	–	–	–	–
Neonates (during NICU stage)													
Gender	M	F	F	F	M	F	M	F	M	F	F	F	F
Birth age (week)	25	29^{+4}	29^{+6}	30^{+6}	24^{+2}	28^{+2}	30^{+1}	25^{+1}	33^{+4}	33^{+2}	23^{+5}	28^{+6}	27^{+2}
Birth weight (g)	400	870	890	1250	320	880	1130	530	1400	1950	350	950	720
pH of UA blood	7.32	7.20	7.30	7.21	7.35	7.25	7.32	7.15	7.30	7.24	7.22	7.10	7.30
Apgar score (1′/5′/10′)	7/9/9	9/9/9	8/9/9	1/7/8	6/7/9	5/7/8	7/9/9	6/9/9	8/10/10	8/10/10	Died[#]	6/7/8	6/8/8
SIMV mode ventilation (days)	49	7	7	1	13	4	6	16	0	0	–	21	23
Perinatal complications[$]	No	No	No	No	Died[*]	No	AIS	No	No	AIS	–	MO[&]	RDS II°; SIRS

[$:+] Denotes days. AIS: amniotic infection syndrome. RDS: respiratory distress syndrome. SIRS: systemic inflammatory response syndrome. SIMV: synchronized intermittent mandatory ventilation. [$]Preterm-related RDS I° and hyperbilirubinemia were not mentioned.
[*] Newborn died with late-onset sepsis.
[#] Newborn died within half an hour after birth.
[&] Meconium Obstruction requiring surgical intervention.

observed in recovery phase in patients with or without H.E.L.P. apheresis.

Mortality was 1/9 in neonates from apheresis-treated mothers and 1/4 in neonates from mothers not treated with the apheresis (Table 3). Eight neonates from nine apheresis-treated preeclamptic mothers were discharged in good clinical conditions.

3.3. Reduction of circulating parameters by H.E.L.P. apheresis

Each apheresis reduced circulating levels of triglycerides, total-, VLDL-, LDL-, HDL-cholesterol and Lp(a) on average by 41%, 35%, 48%, 44%, 11% and 49%, respectively. Plasma viscosity was reduced on average by 12% per session. TNF α, sVCAM-1, E-selectin were reduced by 65%, 23% and 20% respectively. Even though a trend of reduction of ET-1 was noted, this was statistically insignificant. A slight but significant increase in TNF r55 and sICAM-1 was observed. Procoagulatory markers such as homocysteine, fibrinogen and hs-CRP were reduced by 18%, 54% and 50%, respectively. Tissue factor showed a slight but statistically insignificant reduction (Table 4).

3.4. Turnover of fibrinogen, LDL and Lp(a) after H.E.L.P. apheresis

Apstein et al. have previously shown that rate of return of cholesterol after cessation of apheresis to pre-apheresis levels follows equation: $\ln[(\mathrm{Conc}_0 - \mathrm{Conc}_t)/(\mathrm{Conc}_0 - \mathrm{Conc}_{min})] = -kt$, where k is a first order disappearance constant and equivalent to fractional catabolic rates (FCR) [15]. The synthetic rate (SR) can be estimated as a product of FCR and plasma pool size expressed in per kilogram of body weight. FCRs for fibrinogen, LDL and Lp(a) are in accordance to the data described previously [16,17]. The data for SRs need caution because total plasma volume in late gestation may not be comparable to that in healthy subjects. However patients with high initial fibrinogen and Lp(a) values showed higher SRs (Table 5).

3.5. Adverse events

Potential adverse events of H.E.L.P. apheresis include hypotension, vasovagal reaction and gastrointestinal pain. Incidences vary from 0.3% to 2.5% [12]. None of these events were observed during the

Table 4
Reduction of markers by H.E.L.P. apheresis (totally 31 treatments) in 9 preeclamptic patients

Parameters	Pre-apheresis	Post-apheresis	Reduction[a] (%)	P
Lipid and lipoprotein (mmol/L)				
Triglycerides	2.4 (1.1–5.0)	1.4 (0.7–3.3)	−41	<0.001
Total cholesterol	5.9(3.3–7.7)	3.7(2.3–5.6)	−36	<0.001
VLDL-cholesterol	0.8 (0.3–1.8)	0.4 (0.3–0.7)	−48	<0.001
LDL-cholesterol	3.3 (1.6–4.9)	1.8 (0.8–3.4)	−44	<0.001
HDL-cholesterol	1.6 (0.7–2.1)	1.5 (0.7–1.8)	−11	<0.001
Lipoprotein (a) (mg/dL)	37 (8–214)	21 (0–92)	−49	<0.001
Procoagulatory and proinflammatory factors (ng/mL)				
Homocysteine (μmol/L)	5.3 (3.8–8.0)	4.5 (3.2–6.1)	−18	<0.001
Fibrinogen (g/L)	4.3 (1.6–6.4)	2.2 (0.9–3.9)	−54	<0.001
Hs-CRP (mg/L)	10.5 (1.8–5.3)	5.0 (0.8–2.8)	−50	<0.001
Tissue factor (pg/mL)	124 (66–634)	103 (63–788)	−4	NS
TNF-α (pg/mL)	33 (10–96)	16 (0–39)	−65	0.007
TNF r55	2.4 (1.2–7)	2.5 (1.4–9.7)	+16	0.046
sVCAM-1	688 (294–1249)	587 (173–1121)	−19	<0.001
sICAM-1	173 (95–250)	184 (118–281)	+9	0.027
sE-selectin	22.3 (12.5–51.6)	15.5 (9.0–43.7)	−23	0.005
LBP (μg/mL)	10.1 (4.7–17.6)	7.6 (4.7–17.6)	−20	0.018
ET-1 (pg/mL)	0.61 (0–2.64)	0.49 (0–1.42)	−17	NS
Plasma viscosity (mPa s)	1.20 (1.06–1.28)	1.06 (0.93–1.13)	−12	<0.001

[a] Reduction (%) is given as median value, − denote decrease, + denote increase.

Table 5
Turnover data calculated from reaccumulation (6 days) after H.E.L.P. apheresis

	Preeclamptic patients with H.E.L.P. apheresis			
	Nr. 2[a]	Nr. 3	Nr. 4	Nr. 6
Fibrinogen pre-apheresis levels (mg/dL)	490	609	631	374
FCR (Pool day^{-1})	0.165	0.208	0.307	0.188
SR (mg kg^{-1} day^{-1})	36.4	57.0	82.3	29.9
LDL pre-apheresis levels (mg/dL)	–	–	192	154
FCR (Pool day^{-1})	–	–	0.428	0.373
SR (mg kg^{-1} day^{-1})	–	–	34.9	24.4
Lp(a) pre-apheresis levels (mg/dL)	–	–	214	47
FCR (Pool day^{-1})	–	–	0.182	0.205
SR (mg kg^{-1} day^{-1})	–	–	16.5	4.1

FCR: fractional catabolic rate.
SR: synthetic rate.
[a] Patient number as mentioned in Table 3.

treatment in our preeclamptic patients. However, central venous catheter-related adverse event was noticed. In one patient a thrombus was formed around the distal part of the catheter 2 weeks after the placement, but reabsorbed after low dose heparin treatment before delivery without subsequent clinical problems.

4. Discussion

Although the cause remains unclear, preeclampsia may be initiated by alterations in placental perfusion and followed by maternal syndrome. Placenta is believed to be the key component that leads to preeclampsia. Alteration of lipid and lipoprotein profile and metabolism is suggested to be one of the main factors causing compromised placental perfusion. "Acute atherosis" changes was found in placental vasculature bed [18]. Lp(a) was reported markedly increased in preeclampsia compared to normal pregnancy and correlated with the severity of disease [5]. Lp(a), by competing with plasminogen for its binding sites on fibrin clots and on endothelial cells, inhibits its activation to plasmin resulting in reduced fibrinolysis. It also binds and inactivates tissue factor pathway inhibitor favoring thrombotic

processes [19]. Hypertriglyceridemia in preeclampsia might attribute to small dense LDL particles which is susceptible to oxidative modification leading to placental hypoxia and maternal endothelial dysfunction [20]. Abundant evidence of increased lipid or nonlipid oxidative markers have been reported in preeclampsia [21].

We do not intend to compare values of lipid profiles of preeclamptic patients with normal pregnant women at term in this study because lipids values differ drastically at different gestational age and most overt elevations in circulating lipids occur in the last trimester of pregnancy [4,20]. However, FFA was already higher in our preeclamptic patients, which is suggested as one of the toxic factors in preeclampsia [22].

Preeclampsia is also a proinflammatory state superimposed on changes that are present in normal pregnancy due to elevated innate immune and coagulatory system. Circulating levels of proinflammatory cytokines, adhesion molecules, acute phase proteins such as CRP, coagulatory markers such as fibrinogen, sCD40L and tissue factor have been reported to be elevated in preeclamptic women [6,7,23]. This was also proved by our study.

Hypercoagulability is one of the characteristics in preeclampsia. Fibrinogen, a key coagulatory factor and acute phase reactant, plays a central role in thrombosis and strongly modulates hemostasis, plasma viscosity and platelet aggregation [24]. Lipoproteins, together with fibrinogen, is involved in modulation of plasma viscosity. Maintaining lipoproteins and fibrinogen in lower levels should be beneficial in preeclamptic patients because of their high risk profile. Homocysteine stimulates the expression of MCP-1, VCAM-1, and E-selectin [24]. Thus reduction of homocysteine levels may reduce endothelial activation in preeclamptic patients.

We proved that H.E.L.P. apheresis removes circulating factors of TNFα, sVCAM-1, E-selectin, ET-1, LBP and homocysteine besides fibrinogen, CRP and atherogenic lipoproteins in our preeclamptic patients. Simultaneous removal of these factors should improve maternal endothelial function, arrest the progression of proinflammatory and procoagulatory processes, increase placental perfusion by reducing blood viscosity, break the link between placental trigger and maternal syndrome, eventually improve the clinical outcomes.

It is known that till date the only effective treatment of preeclampsia is immediate termination of pregnancy. It is however not an ideal solution since immature fetus delivered preterm has high risk of morbidity and mortality. Women who develop preeclampsia before 33 weeks of gestaion have several fold higher risk for mortality compared to those who develop it at term. For each week of completed gestation in hypertensive women, 50% survival rate is related to birth weight. By 27 weeks, all infants weighing more than 600 g after hypertensive pregnancy have more than 50% chance of intact survival [1,25]. Thus prolongation of gestational age which improves perinatal outcomes but does not compromise the mothers deserves to be considered. The nine preeclamptic patients undergoing H.E.L.P. apheresis differed in their clinical courses and laboratory findings. Even though apheresis procedure was similar, yet the frequency and number of treatments need individualized approach. A decision of performing a next session was based on fibrinogen levels, Doppler indexes and patient's compliance.

There are a few reports describing the continuation of LDL-apheresis during the course of pregnancy in homozygous familial hypercholesterolemic women (37–39 weeks) [26–28]. No adverse cardiac events or placental insufficiency was noted in these patients. Probably underlying mechanisms for safe and successful pregnancy in these patients are similar to those described in this study.

Our study has limitations. The grouping of preeclamptic patients was not randomized and also limited by the small number of patients due to the properties of a pilot study. A randomized controlled trial with a larger cohort is needed to prove the clinical outcomes. Follow-up of newborns from preeclamptic mothers treated with H.E.L.P. apheresis is also needed to exclude long term side effects.

Acknowledgements

Dr. Y. Wang was supported by a grant of German Society of Clinical Chemistry and Laboratory Medicine. We thank Dr. Beate Jaeger for her stimulating discussion and help in preparation of the study protocol.

References

[1] Sibai BM. Prevention of preeclampsia: a big disappointment. Am J Obstet Gynecol 1998;179:1275–8.
[2] Antoinette CB, van Geijn HP, Gustaaf AD. Management and monitoring of severe preeclampsia. Eur J Obstet Gynecol Reprod Biol 2001;96:8–20.

[3] Redman CWG, Sargent IL. Pre-eclampsia, the placenta and the maternal systemic inflammatory response – a review. Placenta 2003;24:S21–7.
[4] Potter JM, Nestel PJ. The hyperlipidemia of pregnancy in normal and complicated pregnancies. Am J Obstet Gynecol 1979;133:165–70.
[5] Wang J, Mimuro S, Lahoud R, Trudinger B, Wang XL. Elevated levels of lipoprotein (a) in women with preeclampsia. Am J Obstet Gynecol 1998;178:146–9.
[6] Vince GS, Starkey PM, Austgulen R, Kwiatkowski D, Redman CWG. Interleukin-6, tumor necrosis factor and soluble tumor necrosis factor receptors in women with preeclampsia. Br J Obstet Gynaecol 1995;102:20–5.
[7] Heyl W, Handt S, Reister F, Gehlen J, Schröder W, Mittermayer C, et al. Elevated soluble adhesion molecules in women with pre-eclampsia: do cytokines like tumor necrosis factor-α and interleukin-1β cause endothelial activation? Eur J Obstet Gynecol Reprod Biol 1999;86:35–41.
[8] Dechend R, Homuth V, Wallukat G, Kreuzer J, Park JK, Theuer J, et al. AT(1) receptor agonistic antibodies from preeclamptic patients cause vascular cells to express tissue factor. Circulation 2000;101:2382–7.
[9] Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med 1998;338:1042–50.
[10] Seidel D, Armstrong VW, Schuff-Werner P, for the HELP Study Group. The HELP-LDL-apheresis multicenter study, an angiographically assessed trial on the role of LDL-apheresis in the secondary prevention of coronary heart disease. I. Evaluation of safety and cholesterol-lowering effects during the first 12 months. Eur J Clin Invest 1991;21:375–83.
[11] Wang Y, Blessing F, Walli AK, Überfuhr P, Fraunberger P, Seidel D. Effects of heparin-mediated extracorporeal low-density lipoprotein precipitation beyond lowering proatherogenic lipoproteins – reduction of circulating proinflammatory and procoagulatory markers. Atherosclerosis 2004;175: 145–50.
[12] Blessing F, Wang Y, Nagel D, Seidel D. The efficacy and safety of the new heparin-induced extracorporeal low-density lipoprotein precipitation system (Plasmat Futura) in comparison with the currently used system (Plasmat Secura). Ther Apher Dial 2004;8:31–6.
[13] Havel RJ. The abnormal lipoprotein of cholestasis. N Engl J Med 1971;285:578–9.
[14] Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350:672–83.
[15] Apstein CS, Zilversmit DB, Lees RS, George PK. Effect of intensive plasma-pheresis on the plasma cholesterol concentration with familial hyper-cholesterolemia. Atherosclerosis 1978;31:105–15.
[16] Armstrong VW, Schleef J, Thiery J, Muche R, Schuff-Werner P, Eisenhauer T, et al. Effect of HELP-LDL-apheresis on serum concentrations of human lipoprotein(a): kinetic analysis of the post-treatment return to baseline levels. Eur J Clin Invest 1989;19:235–40.
[17] Thiery J, Ivandic B, Bahlmann G, Walli AK, Seidel D. Hyperlipoprotein(a)-aemia in nephrotic syndrome. Eur J Clin Invest 1996;26:316–21.
[18] Brosens I, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of pre-eclampsia. Obstet Gynecol Annu 1972;1:177–91.
[19] Caplice NM, Panetta C, Peterson TE, Klepper LS, Mueske CS, Kostner GM, et al. Lipoprotein (a) binds and inactivates tissue factor pathway inhibitor: a novel link between lipoproteins and thrombosis. Blood 2001;98:2980–7.
[20] Sattar N, Bendomir A, Berry C, Shepherd J, et al. Lipoprotein subfraction concentrations in preeclampsia: pathogenic parallels to atherosclerosis. Obstet Gynecol 1997;89:403–8.
[21] Roberts JM, Pearson G, Cutler JA, Lindheimer MD. National Heart Lung and Blood Institute. Summary of the NHLBI working group on research on hypertension during pregnancy. Hypertens Pregnancy 2003;22:109–27.
[22] Metzger BE, Ravnikar V, Vileisis RA, Freinkel N. "Accelerated starvation" and the skipped breakfast in late normal pregnancy. Lancet 1982;1:588–92.
[23] Greer IA, Lyall F, Perera T, Boswell F, Macara LM. Increased concentrations of cytokines, interleukin-6 and interleukin-1 receptor antagonist in plasma of women with preeclampsia: a mechanism for endothelial dysfunction? Obstet Gynecol 1994;84:937–40.
[24] Schuff-Werner P, Schutz E, Seyde WC, Eisenhauer T, Janning G, Armstrong VW, et al. Improved haemorheology associated with a reduction in plasma fibrinogen and LDL in patients being treated by heparin-induced extracorporeal LDL precipitation (HELP). Eur J Clin Invest 1989;19:30–7.
[25] Lee SK, McMillan DD, Ohlsson A, Pendray M, Synnes A, Whyte R, et al. Variations in practice and outcomes in the Canadian NICU network: 1996–1997. Pediatrics 2000;106: 1070–9.
[26] Kroon AA, Swinkels DW, van Dongen PWJ, Stalenhoef AFH. Pregnancy in a patient with homozygous familial hypercholeterolemia treated with long-term low density lipoprotein apheresis. Metabolism 1994;43:1164–70.
[27] Teruel JL, Lasuncion MA, Navarro JF, Carrero P, Ortuno J. Pregnancy in a patient with homozygous familial hypercholesterolemia undergoing low-density lipoprotein apheresis by dextran sulfate adsorption. Metabolism 1995;44:929–33.
[28] Cashin-Hemphill L, Noone M, Abbott JF, Waksmonski CA, Lees RS. Low-density lipoprotein apheresis therapy during pregnancy. Am J Cardiol 2000;86:1160.