Isolated microparticles, but not whole plasma, from women with preeclampsia impair endothelium-dependent relaxation in isolated myometrial arteries from healthy pregnant women

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OBJECTIVE: This study was performed to establish whether microparticles from plasma of women with preeclampsia cause endothelial dysfunction, as described for isolated myometrial arteries in preeclampsia.
STUDY DESIGN: Myometrial arteries were isolated from biopsy specimens obtained at cesarean delivery from healthy pregnant women (n = 22) and mounted in a wire myograph. Bradykinin concentration-response curves were obtained before and after 1-hour incubation or after overnight incubation with one of the following preparations of plasma from individual women with preeclampsia (n = 16): whole plasma, microparticle-free plasma, isolated microparticles resuspended in physiologic saline solution or physiologic saline solution. Overnight incubation was also performed with microparticles isolated from healthy pregnant women (n = 6). One-hour incubation was performed with 2% or 10% solution and overnight incubation with 5% solution.
RESULTS: No effect of preeclamptic plasma, with or without microparticles, on bradykinin-mediated relaxation was observed. Overnight, but not 1-hour, incubation with preeclamptic microparticles caused abolishment of bradykinin-mediated relaxation in contrast to healthy pregnant microparticles ($P < .005$).
CONCLUSION: Preeclamptic microparticles, but not healthy pregnant microparticles cause endothelial dysfunction in isolated myometrial arteries from healthy pregnant women after overnight incubation, whereas other preeclamptic plasma constituents protect the endothelium from this effect. (Am J Obstet Gynecol 2002;187:1686-93.)

Key words: Isolated myometrial arteries, preeclampsia, plasma, microparticles, endothelial function

The central feature in the pathophysiologic mechanisms that result in preeclampsia is a generalized vascular dysfunction. This vascular dysfunction is characterized by endothelial cell activation and resultant expression of factors favoring coagulation and impairment of endothelium-dependent dilatation.[1] These vascular abnormalities are considered to arise from factors derived from the placenta, which circulate in the maternal blood.

Many in vitro experiments have indicated the presence of such factors in the plasma and serum from women with preeclampsia, but their nature is still unclear. Studies that used cultured endothelial cells have shown that plasma or sera from women with preeclampsia can alter endothelial cell function and cause cell activation.[2] Increased cellular permeability, intracellular accumulation of triglycerides, increased thrombomodulin release, increased prostacyclin and nitric oxide production, and increased adhesion molecule expression and nuclear factor-κB activity[3] are reported consequences of incubation with preeclamptic plasma or serum.[2] Studies on isolated arteries reported impairment of bradykinin-induced relaxation already after 1 hour of incubation with 2% pooled preeclamptic plasma.[4,5] It is likely that there is more than one responsible factor; a high molecular weight, lipoprotein-containing fraction in preeclamptic plasma stimulated nitric oxide production in cultured endothelial cells[6] and diminished bradykinin-mediated relaxation in isolated myometrial arteries,[7] whereas a small, aqueous, low-molecular-weight fraction in the preeclamptic plasma stimulated prostacyclin production in cultured endothelial cells.[6] It was proposed that vascular endothelial growth factor (VEGF) may be responsible because anti-VEGF could block the effect of preeclamptic

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plasma on prostacyclin production by cultured endothelial cells and on bradykinin-mediated relaxation in isolated myometrial arteries.[2]

We propose that microparticles may be involved in the generation of endothelial cell dysfunction in preeclampsia. Microparticles are small membrane fragments, released from cells during activation or apoptosis that are capable of altering endothelial cell function through several mechanisms, which could be relevant to preeclampsia. Specifically, microparticles can induce cyclooxygenase expression and prostacyclin production by endothelial cells,[8] up-regulate adhesion molecules on the endothelial surface,[9,10] initiate cytokine (interleukin-6, interleukin-8)[10] and tissue factor[11] production, cause platelet and neutrophil activation,[12] and increase monocyte adhesiveness.[9,11] Recently we reported that the major part of microparticles circulating in the blood of women with preeclampsia or healthy pregnancy is derived from platelets. Furthermore, there are microparticles derived from erythrocytes, leukocytes, lymphocytes, and endothelial cells. We found that many microparticles derived from granulocytes and lymphocytes are increased in preeclampsia.[13] Because microparticles as well as plasma or serum from women with preeclampsia induce many alterations in endothelial cell function and composition of the microparticle population is altered in preeclampsia, we hypothesized that microparticles may underlie the development of endothelial cell dysfunction in preeclampsia. The aim of this study was to establish whether microparticles from plasma of women with preeclampsia induce endothelial cell dysfunction in isolated myometrial arteries from healthy pregnant women.

Material and methods

Patients. Plasma samples were collected in the Academic Medical Center after approval by the medical ethical committee. After informed consent was obtained, plasma samples were collected from women with preeclampsia, who were selected on basis of the criteria as described by the International Society for the Study of Hypertension in Pregnancy: diastolic blood pressure of 110 mm Hg or more on any occasion or 90 mm Hg or more on two separate occasions at least 2 hours apart and proteinuria of 1 g or 2+ stick in two clean midstream urine samples taken at least 4 hours apart or 0.3 g protein per 24 hours or 1+ stick in a sample of specific gravity <1.03, pH <8 developing after 20 weeks' gestation and returning to normal values within 3 months after delivery.[14] Plasma samples were also collected from healthy pregnant women with singleton pregnancies, who were matched for age and gestational age with women with preeclampsia, of whom the blood was used for overnight incubation.

Collection of blood samples. Blood samples were taken from the antecubital vein without tourniquet through a 19-gauge butterfly needle with a Vacutainer blood-collecting system (Becton Dickinson, Rutherford, NJ) into a 4.5-mL tube containing 0.105 mol/L citrate. Blood samples were processed immediately at room temperature to prevent any cell activation. Cells were removed by centrifugation for 20 minutes at 1550 × *g* at room temperature to obtain plasma. Plasma samples were divided in aliquots of 1 mL, snap frozen in liquid nitrogen to preserve the microparticle structure, and stored at –80°C. Before the start of the experiments, the frozen plasma aliquots were thawed on melting ice. Two tubes containing 250 μL plasma were centrifuged at 17570 × *g* for 30 minutes to pellet the microparticles. Subsequently, 225 μL of the microparticle-free plasma was removed from each tube and the microparticle pellet was resuspended in the remaining 25 μL of plasma and 225 μL of calcium-free physiologic saline solution. As previously confirmed, the fraction isolated from plasma according to the described protocol contains microparticles.[13] Whole, microparticle-containing plasma, microparticle-free plasma, and isolated microparticles were dissolved in calcium-free physiologic saline solution to the desired concentration and used immediately. Calcium-free physiologic saline solution was used to prevent fibrin generation in the solutions. To the solutions that were used for overnight incubation, heparin (1 IU/mL) was added.

Solutions. Physiologic saline solution composition (in millimoles per liter) was sodium chloride 119, potassium chloride 4.7, calcium chloride 2.5, magnesium sulfate 1.17, sodium bicarbonate 25, potassium phosphate 1.18, EDTA 0.026, D(+)glucose 5.5, and HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid) 5, pH 7.4. Norepinephrine, L-arginine vasopressin, and bradykinin were all obtained from Sigma-Aldrich (Stockholm, Sweden) and prepared as stock solutions, which were further diluted in physiologic saline solution to the required concentrations.

Myometrial biopsy specimens. Myometrial biopsy specimens were obtained at the Huddinge University Hospital after approval by the medical ethical committee. After obtaining informed consent, uterine biopsy samples were obtained from healthy pregnant women, who were undergoing an elective cesarean delivery at term. At cesarean delivery, after the delivery of the placenta, a full-thickness biopsy specimen of the myometrium was taken from the upper margin of the uterine incision. The site of the biopsy was never the site of the placenta location. The biopsy specimens were collected into ice-cold physiologic saline solution. Small arteries of about 200 to 600 μm in diameter and with a length of approximately 2 mm were immediately dissected from healthy myometrium in the biopsy specimen in ice-cold physiologic saline solution. From each biopsy specimen, four or more arteries of comparable size and preferably from the same artery segment were dissected.

Wire myography. Arteries were mounted on stainless steel wires, 40 μm in diameter, which were attached to a force transducer and a micrometer, respectively, in the organ baths of a four-channel Multi Myograph (model 610, Danish Myo Technology, Denmark), which were filled with physiologic saline solution. Vessel length was measured with a calibrated eyepiece micrometer under the microscope. After all arteries were mounted, they were allowed to equilibrate for 30 minutes at 37°C, while continuously being oxygenated with 5% carbon dioxide in oxygen. All solutions, including the incubation solutions, were refreshed every 30 minutes. A standardized normalization procedure was then performed to allow for calculation of the artery diameter at which the in vivo transmural pressure of the relaxed artery would have been 100 mm Hg. Arteries were then set at 0.9 times this diameter because it is generally accepted that this is the diameter that enables optimal contractile ability for the arteries with a low resting tension. Myodac software was used for these calibrations and for data registration (version 2.1, Danish Myo Technology, Denmark).

After the normalization procedure, the arteries were left to equilibrate for 20 minutes and a reference constriction was then elicited with physiologic saline solution containing 124 mmol/L potassium and 1 μmol/L norepinephrine. After a washout period of 15 minutes, constriction was induced with increasing concentrations of vasopressin (0.1 to 10 nmol/L) until a stable constriction was achieved to approximately the level of the reference constriction. A concentration-response curve with increasing concentrations of bradykinin (1 nmol/L to 3 μmol/L) was then performed. After a washout period, incubation was started with a 2% or a 10% solution of (1) whole preeclamptic plasma, (2) microparticle-free preeclamptic plasma, (3) isolated preeclamptic microparticles resuspended in physiologic saline solution, or (4) physiologic saline solution. Each incubation solution was prepared from the plasma of one woman with preeclampsia. After a 1-hour incubation, the plasma was washed out of the organ bath by flushing three times with physiologic saline solution. The preconstriction was then repeated up to the same concentration of vasopressin as had been used before the incubation, followed by the bradykinin concentration-response curve.

A separate group of arteries was freshly dissected and then incubated overnight at 4°C in 5% solutions of (1) whole preeclamptic plasma, (2) microparticle-free preeclamptic plasma, (3) isolated preeclamptic microparticles resuspended in physiologic saline solution, (4) isolated healthy pregnant microparticles resuspended in physiologic saline solution, or (5) physiologic saline solution. The next morning these arteries were mounted in the wire myograph and normalization, reference constriction, vasopressin preconstriction, and bradykinin concentration-response curve were performed, as described previously.

Data analysis. The force developed by the artery per millimeter of artery segment during application of a certain concentration of a vasoactive substance was calculated with Myodata (Danish Myo Technology, Denmark). Data were then transferred to STATISTICA (version 5.5, StatSoft, Uppsala, Sweden), in which all analyses were performed. All measurements were corrected for the baseline force developed by the arteries. Vasopressin constriction was calculated as the percentage of the reference constriction. Vessel relaxation to bradykinin was calculated as the percentage of vasopressin preconstriction. Analysis of variance (ANOVA) was used to test for differences between groups in diameter, reference, and vasopressin constriction. Paired Student *t* tests were used to compare vasopressin preconstriction before and after incubation. Multivariate analysis of variance for repeated measures (MANOVA) was used to compare bradykinin concentration-response curves before and after incubation and for differences between groups. All data are presented as mean ± SEM. $P < .05$ was considered to be statistically significant.

Results

Patients. Plasma was obtained from 16 women with preeclampsia at admission into the hospital and from 6 healthy pregnant women. Demographic data of these patients at the moment of sampling and at delivery are presented in Table I. Three women with preeclampsia also fulfilled the criteria for the HELLP (hemolysis, elevated liver enzymes, low platelets) or ELLP syndrome[15] at the time of sampling and 1 woman had eclampsia develop. In 4 women with preeclampsia, perinatal death occurred.

Myometrial biopsy specimens were obtained from 22 healthy pregnant women. Demographic data and the indications for cesarean deliveries of these women are presented in Table II.

Arteries. Ninety-two arteries were dissected from the biopsy specimens, usually at least four per biopsy. Six arteries were excluded for having an insufficient endothelial function at the start of the experiments (maximal relaxation to bradykinin <30%). In the 1-hour incubation group, 13 arteries were incubated with physiologic saline solution, 15 with whole preeclamptic plasma (2%, n = 7; 10%, n = 8), 15 with microparticle-free preeclamptic plasma (2%, n = 7; 10%, n = 8), and 14 with isolated preeclamptic microparticles (2%, n = 7; 10%, n = 7). For the overnight incubation, six arteries were incubated with 5% whole preeclamptic plasma, six with 5% microparticle-free preeclamptic plasma, six with 5% isolated preeclamptic microparticles, six with isolated healthy pregnant microparticles, and five with physiologic saline solution. The mean diameter of all arteries was 386.0 ± 19.0 μm. There were no differences

Table I. Demographic data at the moment of plasma collection and at delivery of women with preeclampsia and healthy pregnant women from whom plasma was collected

Sampling	*Preeclampsia*	*Healthy pregnant*
No.	16	6
Age (y)	30.9 ± 1.4	29.9 ± 1.8
Gestational age (wk)	29.7 ± 0.9	29.0 ± 2.5
Parity	0 (0-4)	0.5 (0-1)
Systolic blood pressure (mm Hg)	157.6 ± 4.6	103.3 ± 4.0
Diastolic blood pressure (mm Hg)	100.7 ± 2.4	55.0 ± 4.1
Proteinuria (g/24 h)	3.5 ± 0.7	—
Delivery		
Gestational age (wk)	31.4 ± 0.9	40.4 ± 1.0
Birth weight (g)	1202 ± 163	3622 ± 248

Table II. Demographic data of the healthy pregnant women from whom myometrial biopsy specimens were obtained at cesarean delivery and indication for cesarean delivery

Demographic data		*Indications for cesarean delivery*	
No.	22	Previous cesarean delivery	11
Age (y)	32.3 ± 1.5	Social	6
Gestational age (wk)	38.8 ± 0.2	Previous sphincter damage	4
Parity	1 (0-3)	Breech presentation	1

in diameter of the arteries allocated to the different study groups.

Contractile properties. The mean reference constriction in all arteries was 3.7 ± 0.2 mN/mm. There were no differences in reference constriction between the arteries allocated to the 1-hour incubation groups and the overnight incubation groups. All arteries constricted in response to increasing concentrations of vasopressin. The vasopressin concentration required to get a stable constriction up to the level of the reference constriction in arteries used for 1-hour incubation was usually 1 nmol/L (n = 30) or 0.3 nmol/L (n = 20). Lower (0.1 nmol/L [n = 3]) and higher concentrations (3 nmol/L [n = 9]) were needed in some arteries. The vasopressin preconstriction was similar before and after 1-hour incubation in all arteries (data not shown), except those incubated with whole preeclamptic plasma (105% ± 3% vs 90% ± 4%, $P = .02$ for 2% plasma and 121% ± 6% vs 109% ± 5%, $P = .007$ for 10% plasma). After overnight incubation, the vasopressin concentration used to reach the level of the reference constriction was 0.1 nmol/L (n = 15) or 0.3 nmol/L (n = 14). The level of vasopressin preconstriction was similar in all groups ($P = .86$).

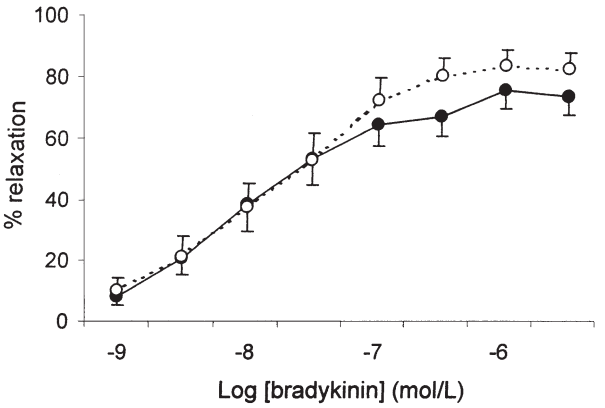

Fig 1. Bradykinin-mediated relaxation before *(closed circles)* and after *(open circles)* 1-hour incubation with physiologic saline solution (n = 13).

Relaxation properties. The arteries incubated for 1 hour with physiologic saline solution relaxed to bradykinin before and after incubation to a similar extent (Fig 1, $P = .61$). There was also no difference in bradykinin-mediated relaxation before and after incubation in arteries incubated with 2% or 10% whole preeclamptic plasma (Fig 2, *A* and *B*, $P = .90$ and $P = .29$, respectively), microparticle-free preeclamptic plasma (Fig 2, *C* and *D*, $P = .56$ and $P = .44$, respectively), or isolated preeclamptic microparticles (Fig 2, *E* and *F*, $P = .85$ and $P = .85$, respectively). Arteries incubated overnight with physiologic saline solution tended to have a reduced relaxation to bradykinin compared with the arteries that were used for 1-hour incubation, although it did not reach significance (maximal relaxation to bradykinin 59% ± 8% [n = 5] vs 80% ± 3% [n = 57], $P = .08$). Bradykinin-mediated relaxation was similar in arteries incubated with isolated healthy pregnant microparticles, whole preeclamptic plasma, or microparticle-free preeclamptic plasma compared with arteries incubated in physiologic saline solution (Fig 3, $P = .54$, $P = .35$, and $P = .98$, respectively). In contrast, bradykinin-mediated relaxation was abolished in arteries incubated overnight with a 5% isolated preeclamptic microparticles solution (Fig 3, $P < .005$ compared with arteries incubated with healthy pregnant microparticles and $P < .0001$ compared with arteries incubated with physiologic saline solution).

Comment

We found no effect of a 1-hour incubation with whole plasma, microparticle-free plasma, or isolated microparticles from individual women with preeclampsia on bradykinin-mediated relaxation in isolated myometrial arteries from healthy pregnant women. However, after an overnight incubation with isolated preeclamptic microparticles bradykinin-mediated relaxation was abol-

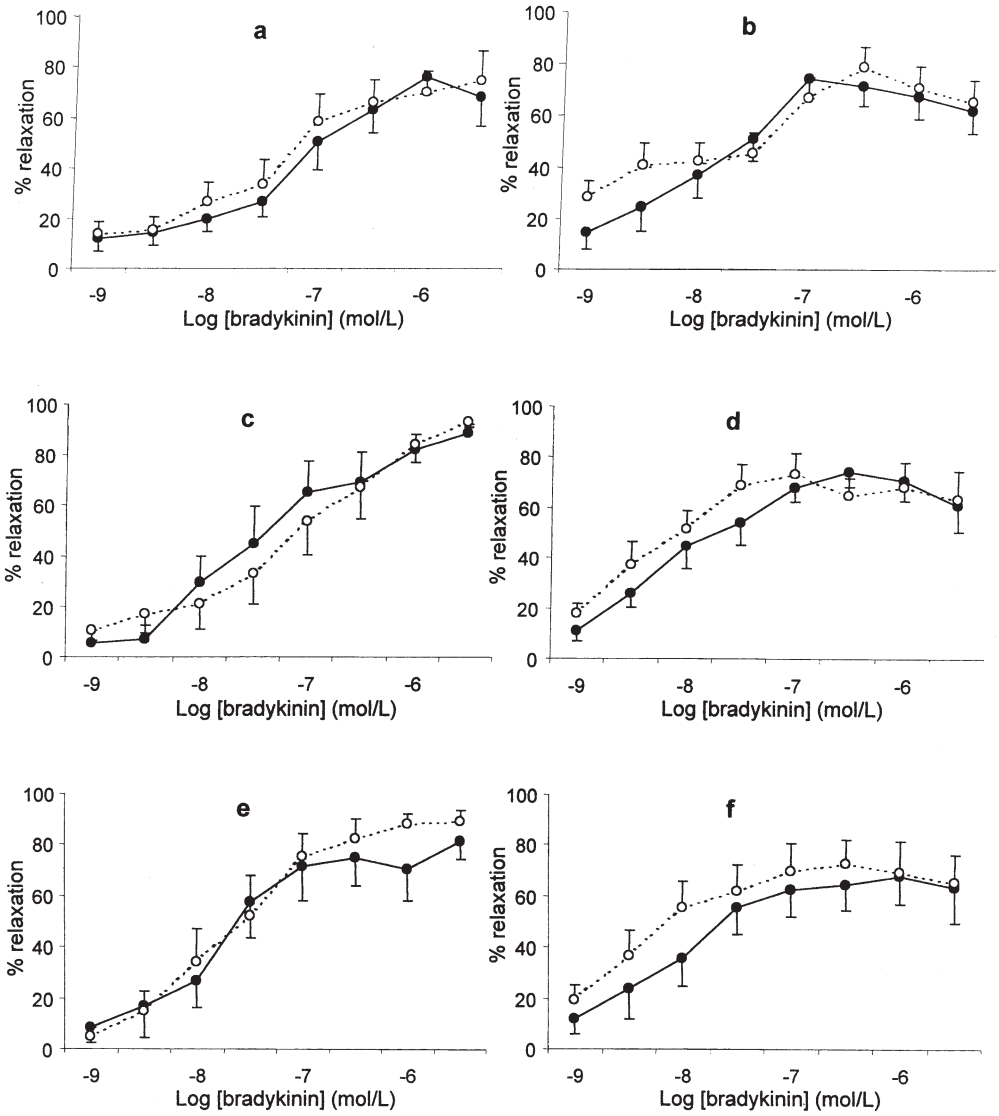

Fig 2. Bradykinin-mediated relaxation before *(closed circles)* and after *(open circles)* 1-hour incubation with 2% (**A,** n = 7) and 10% (**B,** n = 8) whole preeclamptic plasma, 2% (**C,** n = 7), and 10% (**D,** n = 8) microparticle-free preeclamptic plasma and with 2% (**E,** n = 7) and 10% (**F,** n = 7) isolated preeclamptic microparticles in physiologic saline solution.

ished. In contrast, isolated healthy pregnant microparticles did not cause any alterations in bradykinin-mediated relaxation after overnight incubation. Similarly, overnight incubation with whole and microparticle-free preeclamptic plasma had no effect on bradykinin-mediated relaxation. Thus, circulating microparticles from women with preeclampsia in contrast to microparticles from healthy pregnant women are able to damage the endothelium after prolonged exposure, whereas other preeclamptic plasma constituents protect the endothelium from such damage.

We performed two sets of experiments. In the first set, vessels were exposed to microparticle-free preeclamptic plasma, preeclamptic microparticles, or their combination for 1 hour. Previously, a 1-hour incubation with 2% pooled preeclamptic plasma has been shown to be sufficient to demonstrate impaired endothelium-dependent dilation,[4,5] In our study we performed a paired comparison of endothelial function before versus after incubation. Surprisingly, we found no evidence for endothelial dysfunction at all after a 1-hour incubation with 2% plasma or with 10% plasma. We therefore prolonged the incubation period to approximately 18 hours at 4°C in a second set of experiments. In these experiments, vessels were mounted on the wires only after the incubation, which provides the disadvantage that paired observations of endothelial function are no longer possible. However, we considered that quite a large database of responsiveness of freshly isolated vessels was available after the first set of experiments, which could serve as a refer-

ence here. This second set of experiments clearly shows full suppression of endothelium-dependent relaxation after incubation with isolated preeclamptic microparticles, whereas healthy pregnant microparticles, microparticles in preeclamptic plasma, and microparticle-free preeclamptic plasma had no effect. Because microparticle isolation procedures were similar for both types of microparticles, our findings strongly suggest that the endothelial dysfunction developed after incubation with preeclamptic microparticles is specific for preeclamptic microparticles and does not result from alterations in microparticle characteristics during the isolation procedure.

Concerning the mechanism that may cause the endothelial damage, incubation with isolated microparticles caused no endothelial dysfunction after a 1-hour incubation at 37°C, but caused full suppression of bradykinin-mediated relaxation after overnight incubation at 4°C. We believe this indicates that a critical step in the damaging mechanisms of microparticles is of physical rather than biochemical origin because at low temperature enzymatic processes such as free radical production are inhibited to a much greater extent than physical processes such as diffusion and attachment. Thus, the picture emerges that microparticles slowly diffuse toward the endothelium and become attached during overnight incubation. The attached microparticles then induce the endothelial dysfunction either during the incubation period or, more likely, immediately after restoring the temperature to 37°C the next morning after mounting the arteries and before assessment of endothelial function. It cannot be excluded that genetic processes, involved in the regulation of endothelium-dependent relaxation (eg, nitric oxide synthase, cyclo-oxygenase, or endothelin-1) could take place, although the low temperature would slow this process dramatically.

It remains to be investigated which microparticles cause the observed effect on endothelial function. In a previous study we found that granulocyte- and lymphocyte-derived microparticle numbers are increased in preeclampsia. Therefore, it is likely that one or both of these types of microparticles are involved. On the other hand, it is also known that characteristics of microparticles not only depend on the cells they originate from but also strongly on the stimulus that resulted in activation of these cells.[19,20] It does therefore remain possible that microparticles derived from other cell types are responsible for the observed effect.

Although the previously suggested chain of events clearly needs further experimental evidence, preeclamptic plasma had a clear protective effect on the endothelial function. Plasma proteins, in particular albumin, are known to protect endothelial function in isolated arteries.[16] The fact that the isolated microparticle solutions do contain a small amount of plasma, 10 times lower than in the microparticles dissolved in plasma, indicates that the interfering effect of plasma is dependent on the concentration of plasma. An extra argument for this theory is formed by the fact that albumin concentrations are reduced and characteristics of albumin are altered in preeclampsia.[17]

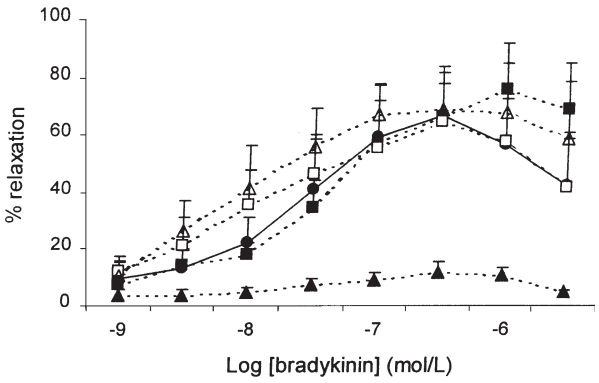

Fig 3. Bradykinin-mediated relaxation after overnight incubation with 5% whole preeclamptic plasma (*solid squares,* n = 6), microparticle-free preeclamptic plasma (*open squares,* n = 6), isolated preeclamptic microparticles in physiologic saline solution (*solid triangles,* n = 6), isolated healthy pregnant microparticles in physiologic saline solution (*open triangles,* n = 6) or with physiologic saline solution (*solid circles,* n = 6).

The question rises whether the damage that we observed is representative for endothelial dysfunction as it occurs in preeclampsia because the time courses of these processes are different. On the basis of this reasoning, a critical step in the damaging effect would be the firm attachment of microparticles to the endothelium. Two conditions in preeclampsia may promote such attachment. First, as already discussed, albumin concentrations are reduced in preeclampsia and albumin structure is altered. Second, oxidative stress is increased in preeclampsia.[1] Oxidative stress has been reported to affect the endothelium protecting layer, resulting in increased adhesion of platelets to the endothelium.[18] This mechanism could well be involved in attachment of microparticles to the endothelium, also because microparticles can express adhesion molecules on their surface.[19]

Furthermore, the vasculature of women who have preeclampsia develop might be more susceptible to damage. This is supported by the fact that preeclampsia occurs more often in women with vascular diseases, such as diabetes or hypertension.[1] Moreover, women who have preeclampsia develop have altered hemodynamic characteristics 5 months after delivery[21] and an increased risk for development of cardiovascular disease later in life.[22]

It remains to be discussed why in our study preeclamptic plasma, with or without microparticles, did not cause endothelial dysfunction in contrast with

previous studies.[4,5] There are, however, some important differences in experimental approach between our study and previous studies. First, we used individual plasma samples instead of pooled plasma to evaluate whether damaging factors are present in all patients. Although the preeclamptic patients included in our study were early and severe cases, with a high incidence of complications, we did not observe any effect of the plasma samples from the 16 preeclampsia patients included in this study on endothelial function. Second, unlike in previous studies, we determined endothelial function before and after a 1-hour incubation in the same artery. This paired observation should provide a more sensitive test for endothelial function changes, but as said before, even with this more sensitive test such changes were not observed after 1-hour incubation. Third, the preconstriction level is essential for the assessment of dilatory function. Supermaximal preconstriction will reduce the ability of the arteries to relax.[23] The vasopressin concentrations that we used for preconstriction were chosen to approximate the high potassium and norepinephrine reference constriction, which are submaximal and were also previously shown to produce a submaximal constriction in isolated myometrial resistance arteries from healthy pregnant women.[24] By using this preconstriction level, we achieved a bradykinin-mediated relaxation up to approximately 80% in all arteries. Interestingly, vasopressin-induced contraction levels were decreased after incubation with whole preeclamptic plasma, but not after incubation with microparticle-free preeclamptic plasma or isolated preeclamptic microparticles. The cause of this effect is unclear, but seems to result from an interaction between plasma and microparticles. Fourth, the plasma that we used in our study was collected in such a way that cellular activation and thus additional microparticle release during sampling and processing was prevented. Furthermore, immediate snap-freezing ensured perseverance of the microparticle structure. Plasma that has not been collected according to this protocol could contain additional, altered, or destructed microparticles that are not present in vivo and that could have a damaging effect on endothelial function.

In conclusion, isolated microparticles from women with preeclampsia cause endothelial dysfunction after overnight incubation in isolated myometrial arteries from healthy pregnant women in contrast to microparticles derived from healthy pregnant women. Furthermore, other preeclamptic plasma constituents protected the endothelium from this damaging effect. We suggest that attachment of microparticles to the endothelium forms a critical step in the introduction of this endothelial dysfunction, whereas albumin and other plasma constituents act protective. This hypothesis will need further investigation.

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