Magnesium sulfate: Rationale for its use in preeclampsia
(endothelium/prostaglandin E1/platelet aggregation/thrombin/hypertension)

KATHLEEN V. WATSON, CHARLES F. MOLDOW, PAUL L. OGBURN, AND HARRY S. JACOB
Departments of Medicine and Obstetrics/Gynecology, University of Minnesota Hospitals, Minneapolis, MN 55455

Communicated by Oscar D. Ratnoff, September 16, 1985

ABSTRACT Preeclampsia is a disorder of pregnancy characterized clinically by hypertension, proteinuria, and edema and characterized pathologically in its late stages by widespread microvascular thrombi. There is evidence from a number of studies that production of prostacyclin (prostaglandin I2, PGI2), a potent vasodilator and inhibitor of platelet aggregation, is deficient in preeclamptic compared to normal pregnancy. Traditional therapy utilizes infusions of large amounts of MgSO4, but the physiologic basis for this is not clear. We studied the effect of MgSO4 on PGI2 release by cultured human umbilical vein endothelial cells (HUVEC) by several methods. By platelet aggregometry, the known antaggregatory effect of intact HUVEC was enhanced by MgSO4. By radiolmmunoassay for 6-keto-PGF1α, the stable metabolite of PGI2, it was shown that MgSO4 amplifies release of PGI2 by HUVEC in a dose-dependent manner, with a peak occurring between 2 and 3 mM. In separate experiments, MgSO4 overcame the enhanced adherence of platelets to HUVEC exhausted by repeated exposure to thrombin. Finally, PGI2 production was 2- to 5-fold greater by HUVEC incubated with plasma obtained from preeclamptic patients undergoing MgSO4 therapy than by HUVEC incubated with pretherapy plasma. We conclude that MgSO4 mediates enhanced production of PGI2 by vascular endothelium, thereby potentially enhancing its thromboresistant properties.

Preeclampsia is a disorder of pregnancy characterized by hypertension, proteinuria, edema, and, in its advanced forms, coagulopathy and seizures (eclampsia). It is a state of uteroplacental vascular insufficiency with grave prognostic implications for mother and fetus. Preeclampsia occurs with an annual incidence in the United States of 7% (1), and worldwide it is estimated yearly to cause five million maternal and fetal deaths (2). In its advanced stages, when biopsied, preeclampsia is characterized pathologically by ballooning of placental and renal endothelial cells and by microvascular occlusions consisting of platelet and fibrin thrombi (3, 4).

Several lines of experimental evidence suggest that prostaglandin production may be relatively deficient during preeclamptic pregnancy. Levels of prostacyclin (prostaglandin I2, PGI2), a potent inhibitor of platelet aggregation and a vasodilator (5), are decreased in the urine (6), amniotic fluid (7), and trophoblastic tissue (8) in preeclamptic pregnancies compared to levels in fluid and tissue samples obtained from normal pregnancies. Furthermore, embolic vesicles from preeclamptic patients synthesize less PGI2-like activity and convert arachidonic acid to PGI2 at a slower rate than those in normal pregnancy (9).

That PGI2 deficiency might be critical to the pathogenesis of preeclampsia is a compelling but unproven concept (10). PGI2 is believed to play an important role in maintenance of thromboresistance at the surface of vascular endothelium by deterring platelet adherence and aggregation (5), and excessive platelet consumption is a well-described feature of preeclampsia. In fact, preeclamptic patients manifest reduced platelet survival (11) and increased platelet activation, detected by elevated levels of circulating platelet-release products (12).

Treatment of preeclampsia has not changed significantly in decades: rest, antihypertensive agents, timely delivery, and parenteral MgSO4 (13). Although MgSO4 has been used successfully to prevent seizures (14), the physiologic basis for the use of large doses of MgSO4 in modern obstetrics remains unclear. Reasoning that diminished production of PGI2 by vascular endothelium might underlie enhanced platelet adhesion, vasoconstriction, and, ultimately, microvascular obliteration—the hallmarks of preeclampsia—we examined the possibility that high levels of MgSO4 might act by promoting synthesis of PGI2 by endothelial cells; if so, the pathological consequences of preeclampsia might be averted. We report that therapeutic levels of magnesium indeed stimulate PGI2 release from cultured endothelial cells and prevent the usual exhaustion of this capability by repetitive thrombin stimulation.

METHODS

Patients. The patients were all under the care of one of us (P.L.O.) and met the following clinical criteria: blood pressure 150/100 on two separate occasions, excretion of 0.1 of protein/liter of random urine specimen, no prior hypertension or renal disease; gestational ages were 33, 33, and 39 weeks. Blood pressure was measured in the hospital, with the patient in the left lateral recumbent position.

Preparation of Endothelial Cells. Human umbilical vein endothelial cells (HUVEC) were grown in culture as described (15). Cells were cultured under 95% air/5% CO2 at 37°C in medium 199 containing 20% fetal bovine serum (GIBCO). They were used at confluence, approximately 5 days after explantation, and were identified as endothelium by their reaction with rabbit antisera to human factor VIII antigen (Boehringer Corporation, New York, NY) (16).

Preparation of Platelets. Platelets were harvested from citrate (0.36%-treated venous blood by centrifugation (400 x g for 5 min) to yield platelet-rich plasma (PRP), which was aspirated into plastic tubes and kept at room temperature. Platelet-poor plasma (PPP) was obtained by centrifugation of the remaining blood (1000 x g for 10 min). Platelet counts were adjusted to 300,000 per mm2 by dilution of PRP with PPP prior to aggregation experiments.

For experiments assessing platelet adherence to cultured endothelium (see below), platelet preparation differed: to wit, platelets were obtained using a modification of the method described by Czervionke et al. (17). Ten parts venous blood mixed with 1 part acid citrate dextrose (ACD) was centri-

Abbreviations: HUVEC, human umbilical vein endothelial cells; PGI2, prostaglandin I2 (prostacyclin); PGF2α, prostaglandin F2α; PPP, platelet-poor plasma; PRP, platelet-rich plasma.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.
fuged (325 × g for 15 min), the resulting PRP was separated by gentle aspiration and recentrifuged (1000 × g for 10 min), and the platelet pellet was resuspended in 10 ml of Tyrode’s solution and 0.4 ml of ACD. After the addition of 250 units of heparin and 100 μCi (1 μCi = 37 kBq) of Na53CrO4 (Amersham), the platelet suspension was incubated at 37°C for 20 min. The platelets then were washed four times as described (17), and the final pellet was suspended in 10 ml of Tyrode’s solution to yield =10¹⁰ platelets per ml. Platelets were used within 4 hr after blood was drawn.

PGI₂ Determined by Bioassay. We used a bioassay for PGI₂ that reflects the ability of endothelial cells to inhibit ADP- or epinephrine-induced aggregation of coincubated platelets. For this, we removed HUVEC from culture dishes by trituration after treating them with trypsin/EDTA (3 min, 37°C). They were then washed three times in Hepes buffer (5.5 mM dextrose/137 mM NaCl/5 mM KCl/10 mM Hepes/l.8 mM CaCl₂, pH 7.35 at 37°C) and suspended in PPP. Endothelial cells in 50 μl of PPP were incubated with PRP (450 μl) and normal saline (0.9% NaCl; 50 μl) in an aggregation module cuvette (Biodata, Hatsboro, PA) at 37°C, with stirring at 1000 rpm for 5 min. Epinephrine or ADP, freshly diluted in normal saline from stock solutions, then was added in a concentration just sufficient to induce platelet aggregation in the absence of endothelial cells. In some experiments, 50 μl of a stock solution of MgSO₄ was added to achieve a final concentration of 3 mM. In selected experiments, the endothelial cell suspensions were preincubated with 250 μM aspirin at 37°C for 45 min and then washed three times before coincubation with platelets. Aggregation was measured as percent light transmission over a minimum of 5 min, and inhibition of aggregation by endothelium (a measure of PGI₂) was defined as the percent decrease in area under aggregation curves over this time period.

PGI₂ Determination by Radioimmunoassay. Cultured HUVEC were incubated with Hepes buffer, as described above, in the presence or absence of 20 μM sodium arachidionate (Sigma). For some experiments, various concentrations of MgSO₄ (1–5 mM) in Hepes buffer were added to the buffer. After incubation for 5 min at 37°C, the cell-free supernatant fluid was aspirated and assayed without extraction for the stable PGI₂ metabolite, 6-keto-PGF₁α, by radioimmunoassay (New England Nuclear). In other experiments, MgSO₄ was replaced by MgCl₂ (1–5 mM), MnSO₄ (1 and 3 mM), or Na2SO₄ (1 and 3 mM). Total cellular protein, measured by the method of Lowry et al. (18), was used to standardize variations in cell number in individual experiments, and results are expressed as concentrations of 6-keto-PGF₁α (ng per dish). Endothelial cell counts were 2.5 x 10⁵ ± 20% per dish.

Human Plasma Experiments. Plasma was obtained from third-trimester preeclamptic patients, from third-trimester normal gravid patients, and from nonpregnant controls. HUVEC monolayers were incubated with these plasmas, diluted 1:1 in Hepes buffer, for 5 min at 37°C as described above. Radioimmunoassays for 6-keto-PGF₁α were performed on unextracted supernatants, with similarly diluted plasmas used to generate standard curves.

Platelet Adherence to Cultured Endothelium. Using a modification of a method of Czervionke et al. (17), we incubated HUVEC monolayers with thrombin (1.0 unit/ml in Hepes buffer) for 5 min at 37°C to stimulate PGI₂ release. The preincubation solution was left on the cells, 0.5 ml of ⁵¹Cr-labeled platelets was added, and the dish then was incubated with rocking (40 times/min, 37°C for 40 min). Some endothelial monolayers, after the initial 5-min incubation with thrombin, underwent a second 5-min incubation with fresh thrombin solution before being exposed to the ⁵¹Cr-labeled platelets as above. Following rocking incubation, nonadherent platelets were removed from the endothelium with multiple additions of buffer, and the nonadherent fractions were pooled. Adherent platelets and their attached endothelial cells were solubilized with 2% Na₂CO₃ in 0.1 N NaOH, and their radioactivity, as well as that of the pooled nonadherent fraction, was measured in a gamma scintillation counter. Percent adherence was calculated by dividing adherent cpm by total cpm added per dish and multiplying by 100. Recovery of total radioactivity (adherent plus nonadherent platelets) averaged 90%.

RESULTS

MgSO₄ Augments HUVEC Capacity to Inhibit Platelet Aggregation. As reported previously (31), endothelial cells inhibit platelet aggregation induced by epinephrine or ADP. As shown in Table 1, inhibition of platelet aggregation is directly proportional to the number of endothelial cells added to PRP. For example, addition of 10⁶ endothelial cells inhibits platelet aggregation by 32%, whereas addition of 10⁵ endothelial cells inhibits aggregation by 98%. The addition to endothelial cells of MgSO₄ (at a final concentration of 3 mM) enhances their capacity to inhibit platelet aggregation. For example, using 2.5 x 10⁶ endothelial cells, the percent inhibition rose, from 14%, to 93% in the presence of 3 mM MgSO₄. Moreover, the augmented inhibition in the presence of 3 mM MgSO₄ was greatest for those concentrations of endothelial cells which alone inhibited platelet aggregation submaximally. This antiaggregatory property of MgSO₄ is due to its effect upon endothelial cells and not upon platelets, as endothelial cells treated with 250 μM aspirin (a concentration that abolishes PGI₂ release, as detected by RIA) did not impair platelet aggregation in the absence or presence of MgSO₄ (Table 1).

MgSO₄ Increases Release of PGI₂ from Endothelium. The inhibitory effect of endothelium on platelet aggregation is generally attributed to PGI₂ synthesis and release by endothelial cells. Using a direct radioimmunoassay that measures the stable PGI₂ end product, 6-keto-PGF₁α, we validated the concept that MgSO₄ augments antiaggregatory effects by promoting PGI₂ release from cultured human endothelial cells. Supplementation of MgSO₄ to levels any higher than physiologic (Fig. 1) increases PGI₂ release by HUVEC in the presence of sodium arachidonate. This statistically significant augmentation (P < 0.005) peaks at a concentration of 3 mM MgSO₄—a level therapeutically sought in the plasmas of preeclamptic patients. A parallel, but diminished, increase in the culture-supernatant levels of 6-keto-PGF₁α is detected for endothelial cells not exposed to sodium arachidonate (Fig. 1). Magnesium ion, and not

Table 1. MgSO₄ enhances the antiaggregatory activity of endothelial cells

<table>
<thead>
<tr>
<th>No. of HUVEC</th>
<th>% inhibition of platelet aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.8 mM Mg²⁺</td>
</tr>
<tr>
<td>Without aspirin</td>
<td></td>
</tr>
<tr>
<td>2.5 x 10⁴</td>
<td>14</td>
</tr>
<tr>
<td>5.0 x 10⁴</td>
<td>29</td>
</tr>
<tr>
<td>1.0 x 10⁵</td>
<td>32</td>
</tr>
<tr>
<td>With aspirin</td>
<td></td>
</tr>
<tr>
<td>(250 μM)</td>
<td></td>
</tr>
<tr>
<td>5.0 x 10⁴</td>
<td>64</td>
</tr>
<tr>
<td>1.0 x 10⁵</td>
<td>98</td>
</tr>
</tbody>
</table>

Platelets were aggregated using standard methods (see Methods) in the presence of various numbers of endothelial cells. The final Mg²⁺ concentration was 0.8 mM (measured in plasma) or 3.0 mM (achieved by the addition of MgSO₄).
MgSO₄.

HUVEC 1-5 mM containing different from tuted for Na₂SO₄ sodium arachidonate. The is endothelial cells and the blood platelets arachidonate (data of adherence is thrombin PGI₂ release.

To Table 2. 

Platelet-Endothelium Adherence. To approximate in vitro the possible effect of MgSO₄ on the interactions between platelets and the blood vessel wall in vivo, we assayed the adherence of platelets to HUVEC in culture. Human endothelial cell monolayers rapidly exhaust their ability to release PGI₂ when repeatedly stimulated with thrombin (19). Thus, platelets normally do not avidly adhere (less than 2%, Fig. 2) to endothelial cells in culture, presumably because of PGI₂ release. Adherence increased significantly ($P < 0.005$) to 11.1% after one thrombin stimulation and 15.3% after two thrombin stimulations (Fig. 2), which others (19) have shown is associated with diminution of endothelial cell PGI₂ synthesis. The addition of MgSO₄ reduces by about half the augmented platelet adherence that follows endothelial exposure to sequential thrombin (Fig. 2); that is, from 11.1 to 6.6% after one stimulation ($P < 0.005$), and from 15.3 to 8.6% after two stimulations ($P < 0.01$). Thus, MgSO₄ appears to replenish or protect the capacity of endothelium to provide PGI₂ activity otherwise exhausted by repeated exposure to thrombin. Its effect is on endothelium and not platelets, as pretreatment of endothelial cells with aspirin, either in the presence or absence of MgSO₄, blocks resistance to thrombin (Fig. 2).

Plasmas from MgSO₄-Treated Patients Increase PGI₂ Release by HUVEC. Plasma obtained from three preeclamptic patients during therapeutic MgSO₄ infusions induced a striking release of PGI₂ when added to cultured HUVEC (Table 3). When compared to release induced by autologous pretreatment plasma, the increase in PGI₂ Release was 2- to 5-fold greater with posttreatment plasma. These increases in PGI₂ in vitro occurred at concentrations of magnesium in plasmas

Table 2. Mg²⁺, and not the counter ion, enhances PGI₂ production by endothelial cells

<table>
<thead>
<tr>
<th>Salt</th>
<th>Conc.</th>
<th>6-Keto-PGF₁α, ng per dish</th>
<th>$P$ value (vs. control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgSO₄</td>
<td>1 mM</td>
<td>8.8 ± 3.8</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3 mM</td>
<td>15.7 ± 0.9</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1 mM</td>
<td>8.4 ± 0.2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3 mM</td>
<td>14.5 ± 1.5</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

HUVEC were incubated with buffer containing sodium arachidonate (20 μM) and either MgSO₄ or MgCl₂. The supernatant was assayed by RIA for 6-keto-PGF₁α. Values are given as mean ± SEM ($n = 3$; three experiments, each done in triplicate). $P$ values (vs. control, no Mg²⁺ or Mn²⁺) were calculated using Student's two-tailed $t$ test.

Table 3. Plasma of MgSO₄-treated preeclamptic patients enhances PGI₂ production by HUVEC

<table>
<thead>
<tr>
<th>Treatment status</th>
<th>6-Keto-PGF₁α, ng per dish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Pre-MgSO₄</td>
</tr>
<tr>
<td></td>
<td>On MgSO₄</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Pre-MgSO₄</td>
</tr>
<tr>
<td></td>
<td>On MgSO₄</td>
</tr>
<tr>
<td>Patient 3</td>
<td>Pre-MgSO₄</td>
</tr>
<tr>
<td></td>
<td>On MgSO₄</td>
</tr>
<tr>
<td>Healthy women</td>
<td>Third trimester</td>
</tr>
<tr>
<td></td>
<td>Nongravid</td>
</tr>
</tbody>
</table>

Cultured endothelial cells were incubated for 5 min with patient or control plasma diluted 1:1 with Heps buffer. The supernatant was then removed and assayed by RIA for 6-keto-PGF₁α. Experiments were done in triplicate but not analyzed statistically. Mean values and ranges (in parentheses) are given.
that closely approximate those yielding maximal PGI2 release from endothelial cells in our buffer system (Fig. 1).

**DISCUSSION**

These results provide a physiologic basis for the use of large amounts of parenteral MgSO4 in this disease. Our data are consistent with the view that MgSO4 amplifies the in vitro generation of PGI2 by HUVEC. Moreover, the magnesium, and not the sulfate, ion enhances PGI2 production, as MgCl2 and MgSO4 are equally stimulatory and neither MnSO4 nor Na2SO4 increases PGI2 release. We suggest that the augmentation of PGI2 release by MgSO4 of PGI2 in the microvasculature could be of significant potential benefit in a disease characterized by disseminated microvascular occlusions, vasoconstriction, and PGI2 deficiency.

Although we cannot provide in vivo data, we noted that the optimal PGI2 response obtained in our in vitro studies was observed at a Mg2+ concentration of 3 mM, which is within the range considered therapeutic in preeclampsia. Moreover, analysis of studies of preeclamptic patients revealed an increase in PGI2 production by HUVEC exposed to their post-MgSO4-therapy plasmas.

This insight into the role of Mg2+ may have ramifications beyond the treatment of preeclampsia. Studies by Altura and coworkers (20, 21) have implicated Mg2+ in mediating resistance to vasoconstriction of coronary and umbilical vessels. Others have hypothesized a role for PGI2 and PGE2 in abrogating the vasoconstrictive effects of angiotensin II and epinephrine (22). Nonetheless, it is to accelerated platelet turnover and microvascular occlusion in the late stages of preeclampsia, rather than vasoconstriction, that PGI2 deficiency may be particularly germane. Hoak et al. (19) have shown that the capacity of endothelium to produce and release PGI2, thereby deterring platelet adherence, is exhausted by repeated exposure to agonists such as thrombin and arachidonic acid (19). Ogburn et al. (23) have shown that plasma levels of free arachidonic acid are higher in preeclampsia than in normal pregnancy. It is conceivable that such bombardment of endothelium by arachidonic acid depletes PGI2 in vivo. If so, our data that pharmacologic levels of MgSO4 tend to preserve thromboresistance of cultured vascular endothelium provides another physiologic basis for the therapeutic use of MgSO4.

The mechanism by which Mg2+ promotes vascular endothelium to produce PGI2 is unclear. Mg2+ is a cofactor in activation of numerous enzymes including phosphorylases (24) and adenylate cyclase and ATPase (25), but it is not known to activate cyclooxygenase or PGI2 synthetase. Ca2+, however, activates phospholipase A2, thereby releasing arachidonic acid substrate for prostaglandin synthesis (26). There is no evidence that Mg2+ is a phospholipase catalyst. Phospholipase A2 activity is regulated by an inhibitory protein, lipomodulin, which is activated by phosphorylation (27). It is possible that Mg2+, paralleling its activation of other phospholipases, influences activity of lipomodulin. If future studies show that high extracellular Mg2+ concentrations can release arachidonic acid from membrane phospholipids, a possible mechanism of its PGI2-stimulating activity would be at hand. In this regard, we note that our studies using plasma from MgSO4-treated patients demonstrated augmented PGI2 release from cultured endothelium without the addition of arachidonic acid.

Another possible mechanism to be pursued involves the companion interactions of Mg2+ and Ca2+ at the membrane and intracellular level. Mg2+ bound to blood vessel endothelium can alter transmembrane Ca2+ fluxes. Furthermore, Mg2+ may compete with Ca2+ for binding sites, thereby displacing Ca2+ and preventing its physiologic effects, such as contraction of smooth muscle (28). In a recent study presented in abstract form (29), Mg2+ infusion stimulated urinary PGI2 excretion in vivo. This effect was antagonized by indomethacin and Ca2+-channel blockers, leading the authors to postulate that Mg2+ infusion alters cellular Ca2+ flux and thereby enhances PGI2 release (29).

Regardless of the mechanism, we note that at least one other disease (thrombotic thrombocytopenic purpura) involving accelerated platelet consumption with microvascular occlusions by platelet aggregates has been postulated to be due to PGI2 deficiency (30). We suggest that MgSO4 may, therefore, be beneficial to these patients also.

We acknowledge the cooperation of the nurses in the Metropolitan Medical Center Labor and Delivery Suite. We thank Libby Sigmon for technical assistance and encouragement and Sue Marshall, Sandra Halberg, Lisa Kepler, and Carol Taubert for assistance in manuscript preparation.