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Magnesium sulfate: Rationale for its use in preeclampsia

(endothelium/prostacyclin/platelet aggregation/thrombin/hypertension)

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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 I_2, PGI_2), a potent vasodilator and inhibitor of platelet aggregation, is deficient in preeclamptic compared to normal pregnancy. Traditional therapy utilizes infusions of large amounts of $MgSO_4$, but the physiologic basis for this is not clear. We studied the effect of $MgSO_4$ on PGI_2 release by cultured human umbilical vein endothelial cells (HUVEC) by several methods. By platelet aggregometry, the known antiaggregatory effect of intact HUVEC was enhanced by $MgSO_4$. By radioimmunoassay for 6-keto-$PGF_{1\alpha}$, the stable metabolite of PGI_2, it was shown that $MgSO_4$ amplifies release of PGI_2 by HUVEC in a dose-dependent manner, with a peak occurring between 2 and 3 mM. In separate experiments, $MgSO_4$ overcame the enhanced adherence of platelets to HUVEC exhausted by repeated exposure to thrombin. Finally, PGI_2 production was 2- to 5-fold greater by HUVEC incubated with plasma obtained from preeclamptic patients undergoing $MgSO_4$ therapy than by HUVEC incubated with pretherapy plasma. We conclude that $MgSO_4$ mediates enhanced production of PGI_2 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 I_2, PGI_2), 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, umbilical vessels from preeclamptic patients synthesize less PGI_2-like activity and convert arachidonic acid to PGI_2 at a slower rate than those in normal pregnancy (9).

That PGI_2 deficiency might be critical to the pathogenesis of preeclampsia is a compelling but unproven concept (10). PGI_2 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 $MgSO_4$ (13). Although $MgSO_4$ has been used successfully to prevent seizures (14), the physiologic basis for the use of large doses of $MgSO_4$ in modern obstetrics remains unclear. Reasoning that diminished production of PGI_2 by vascular endothelium might underlie enhanced platelet adherence, vasoconstriction, and, ultimately, microvascular obliteration—the hallmarks of preeclampsia—we examined the possibility that high levels of $MgSO_4$ might act by promoting synthesis of PGI_2 by endothelial cells; if so, the pathological consequences of preeclampsia might be averted. We report that therapeutic levels of magnesium indeed stimulate PGI_2 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% CO_2 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 × *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 × *g* for 10 min). Platelet counts were adjusted to 300,000 per mm^3 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-

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Abbreviations: HUVEC, human umbilical vein endothelial cell(s); PGI_2, prostaglandin I_2 (prostacyclin); $PGF_{1\alpha}$, prostaglandin $F_{1\alpha}$; PPP, platelet-poor plasma; PRP, platelet-rich plasma.

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 $Na_2{}^{51}CrO_4$ (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 ≈10^8 platelets per ml. Platelets were used within 4 hr after blood was drawn.

PGI_2 Determined by Bioassay. We used a bioassay for PGI_2 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/1.8 mM $CaCl_2$, pH 7.35 at 37°C) and suspended in PPP. Intact 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 $MgSO_4$ 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 PGI_2) was defined as the percent decrease in area under aggregation curves over this time period.

PGI_2 Determination by Radioimmunoassay. Cultured HUVEC were incubated with Hepes buffer, as described above, in the presence or absence of 20 μM sodium arachidonate (Sigma). For some experiments, various concentrations of $MgSO_4$ (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 PGI_2 metabolite, 6-keto-$PGF_{1\alpha}$, by radioimmunoassay (New England Nuclear). In other experiments, $MgSO_4$ was replaced by $MgCl_2$ (1–5 mM), $MnSO_4$ (1 and 3 mM), or Na_2SO_4 (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-$PGF_{1\alpha}$ (ng per dish). Endothelial cell counts were 2.5×10^5 (±20%) per dish.

Human Plasma Experiments. Plasma was obtained from third-trimester preeclamptic patients, from third-trimester normal gravid patients, and from nongravid controls. HUVEC monolayers were incubated with these plasmas, diluted to 1:1 in Hepes buffer, for 5 min at 37°C as described above. Radioimmunoassays for 6-keto-$PGF_{1\alpha}$ 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 PGI_2 release. The preincubation solution was left on the cells, 0.5 ml of ^{51}Cr-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 ^{51}Cr-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% Na_2CO_3 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

$MgSO_4$ 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 10^5 endothelial cells inhibits platelet aggregation by 32%, whereas addition of 10^6 endothelial cells inhibits aggregation by 98%. The addition to endothelial cells of $MgSO_4$ (at a final concentration of 3 mM) enhances their capacity to inhibit platelet aggregation. For example, using 2.5×10^4 endothelial cells, the percent inhibition rose, from 14%, to 93% in the presence of 3 mM $MgSO_4$. Moreover, the augmented inhibition in the presence of 3 mM $MgSO_4$ was greatest for those concentrations of endothelial cells which alone inhibited platelet aggregation submaximally. This antiaggregatory property of $MgSO_4$ is due to its effect upon endothelial cells and not upon platelets, as endothelial cells treated with 250 μM aspirin (a concentration that abolishes PGI_2 release, as detected by RIA) did not impair platelet aggregation in the absence or presence of $MgSO_4$ (Table 1).

$MgSO_4$ Increases Release of PGI_2 from Endothelium. The inhibitory effect of endothelium on platelet aggregation is generally attributed to PGI_2 synthesis and release by endothelial cells. Using a direct radioimmunoassay that measures the stable PGI_2 end product, 6-keto-$PGF_{1\alpha}$, we validated the concept that $MgSO_4$ augments antiaggregatory effects by promoting PGI_2 release from cultured human endothelial cells. Supplementation of $MgSO_4$ to levels any higher than physiologic (Fig. 1) increases PGI_2 release by HUVEC in the presence of sodium arachidonate. This statistically significant augmentation ($P < 0.005$) peaks at a concentration of 3 mM $MgSO_4$—a level therapeutically sought in the plasmas of preeclamptic patients. A parallel, but diminished, increase in the culture-supernatant levels of 6-keto-$PGF_{1\alpha}$ is detected for endothelial cells not exposed to sodium arachidonate (Fig. 1). Magnesium ion, and not

Table 1. $MgSO_4$ enhances the antiaggregatory activity of endothelial cells

No. of HUVEC	% inhibition of platelet aggregation	
	0.8 mM Mg^{2+}	3.0 mM Mg^{2+}
Without aspirin		
2.5×10^4	14	93
5.0×10^4	29	56
1.0×10^5	32	79
2.5×10^5	64	72
1.0×10^6	98	92
With aspirin (250 μM)		
5.0×10^4	5	6
1.0×10^6	2	1

Platelets were aggregated using standard methods (see *Methods*) in the presence of various numbers of endothelial cells. The final Mg^{2+} concentration was 0.8 mM (measured in plasma) or 3.0 mM (achieved by the addition of $MgSO_4$).

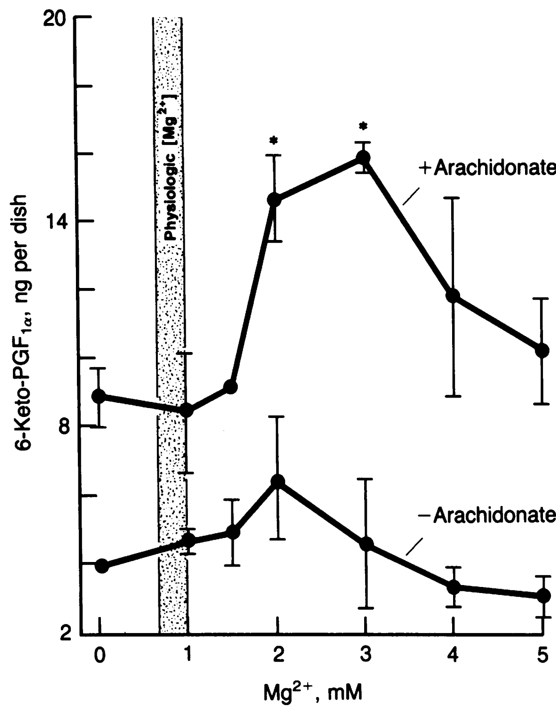

FIG. 1. Stimulation of PGI_2 release by HUVEC exposed to $MgSO_4$. HUVEC were incubated in Hepes buffer (see *Methods*) containing 1–5 mM Mg^{2+}, in the presence or absence of 20 μM sodium arachidonate. The supernatant then was assayed by RIA. *, Significantly different from control ($P < 0.005$, n = 14).

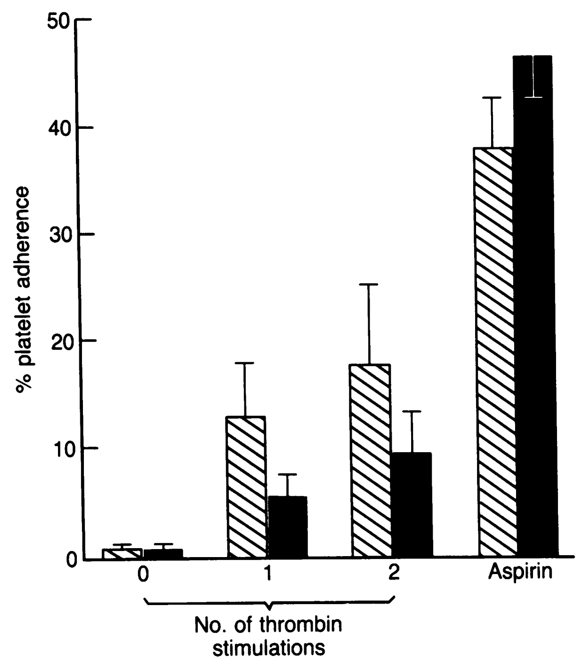

FIG. 2. Effect of Mg^{2+} on platelet adherence to HUVEC *in vitro*. Cultured endothelial cells were exposed once or twice to thrombin (1.0 unit/ml, 5 min), or exposed to aspirin (250 μM, 45 min), in the presence (solid bars) or absence (hatched bars) of 3 mM $MgSO_4$. Adherence of ^{51}Cr-labeled platelets to endothelium was determined by scintillation counting. Data are represented as mean ± SEM (n = 10).

sulfate, is responsible for the increment in PGI_2 release, since almost identical results are obtained when $MgCl_2$ is substituted for $MgSO_4$ (Table 2). Further, neither $MnSO_4$ nor Na_2SO_4 causes an increase in 6-keto-$PGF_{1\alpha}$ release by endothelial cells in the presence or absence of sodium arachidonate (data not shown).

Platelet–Endothelium Adherence. To approximate *in vitro* the possible effect of $MgSO_4$ 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 PGI_2 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 PGI_2 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 PGI_2 synthesis. The addition of $MgSO_4$ 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, $MgSO_4$ appears to replenish or protect the capacity of endothelium to provide PGI_2 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 $MgSO_4$, blocks resistance to thrombin (Fig. 2).

Table 2. Mg^{2+}, and not the counter ion, enhances PGI_2 production by endothelial cells

Salt	Conc.	6-Keto-$PGF_{1\alpha}$, ng per dish	*P* value (vs. control)
$MgSO_4$	1 mM	8.8 ± 3.8	
	3 mM	15.7 ± 0.9	<0.005
$MgCl_2$	1 mM	8.4 ± 0.2	
	3 mM	14.5 ± 1.5	<0.005

HUVEC were incubated with buffer containing sodium arachidonate (20 μM) and either $MgSO_4$ or $MgCl_2$. The supernatant was assayed by RIA for 6-keto-$PGF_{1\alpha}$. Values are given as mean ± SEM ($n = 9$; three experiments, each done in triplicate). *P* values (vs. control, no Mg^{2+} or Mn^{2+}) were calculated using Student's two-tailed *t* test.

Plasmas from $MgSO_4$-Treated Patients Increase PGI_2 Release by HUVEC. Plasma obtained from three preeclamptic patients during therapeutic $MgSO_4$ infusions induced a striking release of PGI_2 when added to cultured HUVEC (Table 3). When compared to release induced by autologous pretreatment plasma, the increase in PGI_2 release was 2- to 5-fold greater with posttreatment plasma. These increases in PGI_2 *in vitro* occurred at concentrations of magnesium in plasmas

Table 3. Plasma of $MgSO_4$-treated preeclamptic patients enhances PGI_2 production by HUVEC

	Treatment status	6-Keto-$PGF_{1\alpha}$, ng per dish
Patient 1	Pre-$MgSO_4$	2.4 (2.0–3.1)
	On $MgSO_4$	12.4 (10.2–13.4)
Patient 2	Pre-$MgSO_4$	6.7 (5.9–7.0)
	On $MgSO_4$	18.0 (17.2–18.9)
Patient 3	Pre-$MgSO_4$	3.5 (2.6–4.2)
	On $MgSO_4$	6.5 (5.8–7.8)
Healthy women		
Third trimester	—	5.2 (4.0–6.0)
Nongravid	—	4.7 (4.0–5.2)

Cultured endothelial cells were incubated for 5 min with patient or control plasma diluted 1:1 with Hepes buffer. The supernatant was then removed and assayed by RIA for 6-keto-$PGF_{1\alpha}$. Experiments were done in triplicate but not analyzed statistically. Mean values and ranges (in parentheses) are given.

that closely approximate those yielding maximal PGI_2 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 $MgSO_4$ in this disease. Our data are consistent with the view that $MgSO_4$ amplifies the *in vitro* generation of PGI_2 by HUVEC. Moreover, the magnesium, and not the sulfate, ion enhances PGI_2 production, as $MgCl_2$ and $MgSO_4$ are equally stimulatory and neither $MnSO_4$ nor Na_2SO_4 increases PGI_2 release. We suggest that the augmentation of PGI_2 release by $MgSO_4$ of PGI_2 in the microvasculature could be of significant potential benefit in a disease characterized by disseminated microvascular occlusions, vasoconstriction, and PGI_2 deficiency.

Although we cannot provide *in vivo* data, we noted that the optimal PGI_2 response obtained in our *in vitro* studies was observed at a Mg^{2+} concentration of 3 mM, which is within the range considered therapeutic in preeclampsia. Moreover, analysis of studies of preeclamptic patients revealed an increase in PGI_2 production by HUVEC exposed to their post-$MgSO_4$-therapy plasmas.

This insight into the role of Mg^{2+} may have ramifications beyond the treatment of preeclampsia. Studies by Altura and coworkers (20, 21) have implicated Mg^{2+} in mediating resistance to vasoconstriction of coronary and umbilical vessels. Others have hypothesized a role for PGI_2 and PGE_2 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 PGI_2 deficiency may be particularly germane. Hoak *et al.* (19) have shown that the capacity of endothelium to produce and release PGI_2, 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 PGI_2 *in vivo*. If so, our data that pharmacologic levels of $MgSO_4$ tend to preserve thromboresistance of cultured vascular endothelium provides another physiologic basis for the therapeutic use of $MgSO_4$.

The mechanism by which Mg^{2+} promotes vascular endothelium to produce PGI_2 is unclear. Mg^{2+} 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 PGI_2 synthetase. Ca^{2+}, however, activates phospholipase A_2, thereby releasing arachidonic acid substrate for prostaglandin synthesis (26). There is no evidence that Mg^{2+} is a phospholipase catalyst. Phospholipase A_2 activity is regulated by an inhibitory protein, lipomodulin, which is activated by phosphorylation (27). It is possible that Mg^{2+}, paralleling its activation of other phosphorylases, influences activity of lipomodulin. If future studies show that high extracellular Mg^{2+} concentrations can release arachidonic acid from membrane phospholipids, a possible mechanism of its PGI_2-stimulating activity would be at hand. In this regard, we note that our studies using plasma from $MgSO_4$-treated patients demonstrated augmented PGI_2 release from cultured endothelium without the coaddition of arachidonic acid.

Another possible mechanism to be pursued involves the companion interactions of Mg^{2+} and Ca^{2+} at the membrane and intracellular level. Mg^{2+} bound to blood vessel endothelium can alter transmembrane Ca^{2+} fluxes. Furthermore, Mg^{2+} may compete with Ca^{2+} for binding sites, thereby displacing Ca^{2+} and preventing its physiologic effects, such as contraction of smooth muscle (28). In a recent study presented in abstract form (29), Mg^{2+} infusion stimulated urinary PGI_2 excretion *in vivo*. This effect was antagonized by indomethacin and Ca^{2+}-channel blockers, leading the authors to postulate that Mg^{2+} infusion alters cellular Ca^{2+} flux and thereby enhances PGI_2 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 PGI_2 deficiency (30). We suggest that $MgSO_4$ may, therefore, be beneficial to these patients also.

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