Antenatal magnesium sulfate treatment and risk of necrotizing enterocolitis in preterm infants born at less than 32 weeks of gestation

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Antenatal magnesium sulfate (MgSO4) treatment is widely used for fetal neuroprotection in women at risk of preterm delivery. However, some studies have recently suggested that in utero MgSO4 exposure is associated with an increased risk of necrotizing enterocolitis (NEC). This study aimed to investigate the association between antenatal MgSO4 treatment and risk of NEC. This retrospective cohort study included 756 infants born at 24–31 weeks’ gestation. Subjects were classified into three groups: period 1, when MgSO4 treatment protocol for fetal neuroprotection was not adopted (n = 267); period 2, when the protocol was adopted (n = 261); and period 3, when the protocol was withdrawn because of concern of risk of NEC (n = 228). Rates of NEC (≥ stage 2b) were analyzed according to time period and exposure to antenatal MgSO4. Significant difference in the rate of NEC was not found across the three time periods (2.6% vs. 6.5% vs. 4.8% in periods 1, 2 and 3, respectively, p = 0.103). The rate of NEC was comparable between the infants unexposed and exposed to antenatal MgSO4 (5.1% vs. 3.6%, p = 0.369). These results showed that antenatal MgSO4 treatment was not associated with risk of NEC in our study population.

Magnesium sulfate (MgSO4) is one of the most commonly used medications in obstetric practice today since its first reported use in 19161,2. Over the past century, MgSO4 has been widely used as an anticonvulsant for treating eclampsia, for preventing eclampsia in women with preeclampsia, and as a tocolysis despite controversy regarding its efficacy1. Moreover, recent evidence has suggested that antenatal MgSO4 may have fetal neuroprotection in women with threatened preterm delivery1. Various randomized controlled trials, systematic reviews, and meta-analyses have revealed that MgSO4 could lead to about 30–40% relative reduction of moderate to severe cerebral palsy and gross motor dysfunction in surviving infants without adverse perinatal outcome5–9. In 2010, the American College of Obstetricians and Gynecologists (ACOG) released a Committee Opinion encouraging MgSO4 administration before anticipated early preterm birth less than 32 weeks of gestation to reduce the risk of cerebral palsy10.

Some studies have recently raised concerns about gastrointestinal effects of antenatal MgSO4 exposure on preterm neonates, including necrotizing enterocolitis (NEC) and spontaneous intestinal perforation (SIP)11,12. However, two larger reports by the Pediatrix Medical Group and the Canadian Neonatal Network showed no increase in NEC or SIP in relationship to any antenatal MgSO4 exposure13,14. Recent meta-analysis and publications also supported these results15,16. As most evidences regarding association of antenatal MgSO4 with gastrointestinal complications have been controversial, the role of antenatal MgSO4 in association with gastrointestinal complications is not well established yet17.

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In our institute, the protocol of antenatal MgSO₄ treatment for fetal neuroprotection was adopted in 2014. However, concern about the risk of neonatal gastrointestinal complications including NEC and SIP has led to treatment withdrawal after two years. Therefore, the objective of this study was to investigate whether these protocol changes might have any effect on the risk of NEC by comparing outcomes according to time periods classified by the adoption and withdrawal of antenatal MgSO₄ treatment protocol for fetal neuroprotection. In addition, we investigated whether antenatal MgSO₄ treatment per se or for fetal neuroprotection was associated with the risk of NEC in preterm neonates at 24‒31 weeks of gestation.

Results

A total of 756 neonates were enrolled in this study, including 267 (35.3%) during period 1, 261 (34.5%) during period 2, and 228 (30.1%) during period 3. Maternal characteristics including maternal age, parity, and body mass index were similar across the three time periods. However, distributions of singleton, twin, and triplet pregnancies were significantly different among the three time periods (Table 1). Rates of antenatal MgSO₄ treatment were significantly different across the three time periods (20.9%, 62.1% and 14.9% in periods 1, 2, and 3, respectively, \( p < 0.001 \)). The most common indication of MgSO₄ treatment during period 2 was fetal neuroprotection whereas that during periods 1 and 3 was severe preeclampsia. A total dose of MgSO₄ used during period 2 was significantly lower than that during period 1 or period 3 because the period of antenatal MgSO₄ treatment for fetal neuroprotection was shorter than those used for severe preeclampsia or tocolysis.

Neonatal outcomes including gestational age at delivery, birth weight, sex, and rate of 1-min Apgar score < 4 were similar across the three time periods. However, the rate of 5-min Apgar score < 7 was significantly lower during period 3 than that during period 1 (Table 2). NEC (≥ stage 2b) occurred in 41 among 756 neonates (5.4%). There was no significant difference in the rate of NEC (≥ stage 2b) across the three time periods (20.9% vs. 62.1% and 14.9% in periods 1, 2, and 3, respectively, \( p = 0.103 \)). The median postnatal age at the onset of NEC was 18.5 days (range, 3–63 days). Early-onset NEC occurred in 14 (40.0%), and late-onset NEC occurred in 21 (60.0%), but there was no significant difference in the age of onset of NEC across the three time periods. Laboratory findings

Table 1. Maternal baseline characteristics and pregnancy outcomes according to time period. Data are presented as number (percentage) or mean ± standard deviation. MgSO₄ magnesium sulfate, PTL preterm labor, PPROM preterm premature rupture of membrane. 1 Significantly different between period 2 and period 3 (Bonferroni test). 2 Significantly different between period 1 and period 2 (Bonferroni test). 3 Significantly different between period 1 and period 3 (Bonferroni test).
of NEC including metabolic acidosis (pH < 7.2) and thrombocytopenia (platelet < 150,000/μL), and radiologic and sonographic findings of NEC including fixed loop or severe ileus, portal venous gas, pneumatosis intestinalis, and ascites were similar across the three time periods. Among 35 neonates with NEC (≥ stage 2b), 23 had NEC perforation which was confirmed by pathologic examination after surgery. The most common site of NEC perforation was jejunum, but the locations of NEC perforation were not significant different across the three time periods. The rate of SIP during period 3 was higher than that during period 1.

Rates of NEC (≥ stage 2b), NEC perforation, and SIP were not significantly different between the infants exposed and unexposed to antenatal MgSO4 (Table 3). And the results were similar in the subgroup analyses stratified by gestational age at delivery (preterm birth less than 26 weeks of gestation and preterm birth at 26–31 weeks of gestation). Rates of NEC (≥ stage 2b), NEC perforation, and SIP were also not significantly different between the infants exposed and unexposed to antenatal MgSO4 treatment. Data are presented as number (percentage), MgSO4, magnesium sulfate, aOR adjusted odds ratio, CI confidence interval, NEC necrotizing enterocolitis, PTB preterm birth, SIP spontaneous intestinal perforation. 1 Adjusted for plurality, antenatal corticosteroids treatment, nifedipine treatment, and gestational age at delivery.

### Table 2. Neonatal outcomes according to time period. Data are presented as number (percentage), mean ± standard deviation or median (range). GA gestational age, NEC necrotizing enterocolitis, SIP spontaneous intestinal perforation. 1 Significantly different between period 1 and period 3 (Bonferroni test).

| Period | GA at delivery (weeks) | Birth weight (kg) | Male | 1-min Apgar score < 4 | 5-min Apgar score < 7 | Neonatal death | NEC (≥ stage 2b) | Stage 2b | Stage 3a | Stage 3b | Onset of NEC (days) | Early-onset | Late-onset | Laboratory findings | Radiology and ultrasound findings |
|--------|------------------------|-------------------|------|----------------------|----------------------|----------------|---------------|----------|---------|---------|------------------|-------------|-------------|---------------------|-----------------------------------|
| Period 1 (n = 267) | 28.3 ± 2.5 | 1.12 ± 0.40 | 145 (54.3) | 38 (14.2) | 59 (22.1) | 8 (3.0) | 7 (2.6) | 2 (28.6) | 1 (14.3) | 4 (57.1) | 21 (3–61) | 3 (42.9) | 4 (57.1) | 6 (85.7) | 7 (100) | 2 (28.6) | 4 (57.1) | 0 (0) | 4 (400) | 1 (0.4) |
| Period 2 (n = 261) | 27.9 ± 2.3 | 1.07 ± 0.38 | 139 (53.3) | 44 (16.9) | 45 (17.2) | 14 (5.4) | 17 (6.5) | 4 (23.5) | 4 (23.5) | 9 (52.9) | 13.5 (3–53) | 8 (47.1) | 13 (76.5) | 6 (85.7) | 17 (100) | 6 (35.3) | 12 (70.6) | 1 (14.3) | 1 (1.1) | 5 (1.9) |
| Period 3 (n = 228) | 28.3 ± 2.3 | 1.09 ± 0.38 | 128 (56.1) | 33 (14.5) | 30 (13.2) | 16 (7.0) | 11 (4.8) | 1 (9.1) | 1 (9.1) | 9 (81.8) | 21 (3–63) | 3 (27.3) | 8 (72.7) | 8 (72.7) | 11 (100) | 3 (27.3) | 8 (72.7) | 0 (0) | 1 (11.1) | 10 (4.4) |

| P value | 0.142 | 0.339 | 0.813 | 0.656 | 0.0331 | 0.119 | 0.103 | 0.591 | 0.0081 |

### Table 3. Neonatal outcomes according to exposure to antenatal MgSO4 treatment. Data are presented as number (percentage), MgSO4 unexposed MgSO4 exposed aOR (95% CI) 1

| MgSO4 unexposed | MgSO4 exposed | P value | aOR (95% CI) 1 |
|------------------|--------------|---------|----------------|
| NEC (≥ stage 2b) | All infants | 26/509 (5.1) | 9/247 (3.6) | 0.369 | 0.70 (0.31–1.55) | 0.373 |
|                  | PTB < 26 weeks | 16/122 (13.1) | 2/50 (4.0) | 0.076 | 0.27 (0.06–1.28) | 0.100 |
|                  | PTB 26–31 weeks | 10/387 (2.6) | 7/197 (3.6) | 0.510 | 1.19 (0.43–3.27) | 0.734 |
| NEC perforation | All infants | 18/509 (3.5) | 5/247 (2.0) | 0.256 | 0.58 (0.21–1.64) | 0.306 |
|                  | PTB < 26 weeks | 13/122 (10.7) | 1/50 (2.0) | 0.059 | 0.18 (0.02–1.47) | 0.109 |
|                  | PTB 26–31 weeks | 5/387 (1.3) | 4/197 (2.0) | 0.494 | 1.32 (0.34–5.08) | 0.691 |
| SIP              | All infants | 9/509 (1.8) | 7/247 (2.8) | 0.340 | 1.82 (0.65–5.10) | 0.252 |
|                  | PTB < 26 weeks | 4/122 (3.3) | 2/50 (4.0) | 1.000 | 1.13 (0.19–6.69) | 0.894 |
|                  | PTB 26–31 weeks | 5/387 (1.3) | 5/197 (2.5) | 0.317 | 2.35 (0.64–8.57) | 0.196 |
of gestation, because the indication of antenatal MgSO₄ treatment protocol for fetal neuroprotection in our
drawal of antenatal MgSO₄ treatment for fetal neuroprotection). We found that protocol changes had no effect
important risk factor is prematurity. The earlier the gestational age at delivery, the higher the risk of NEC25.
in setting of immature intestine along with decreased blood flow to the intestine of preterm neonates8. However,
resistance and reduce mesenteric blood flow24. The hypomotility of intestine is also due to cholinergic effect of
reporting that there is no significant difference in the incidence of NEC between placebo and antenatal MgSO₄
treatment per se or for fetal neuroprotection was associated with the risk of NEC in preterm neonates born at 24—31 weeks of gestation in
this population. These findings are consistent with previous randomized control trials and other recent studies
reporting that there is no significant difference in the incidence of NEC between placebo and antenatal MgSO₄
treatment groups9,13–20.

In this study, we investigated the effect of antenatal MgSO₄ treatment on the risk of NEC by comparing neonatal
outcomes before and after two major changes in the antenatal MgSO₄ treatment protocol (adoption and with-
drawal of antenatal MgSO₄ treatment for fetal neuroprotection). We found that protocol changes had no effect
on the rate of NEC in this population. We also found that neither antenatal MgSO₄ treatment nor for fetal
neuroprotection was associated with the risk of NEC in preterm neonates born at 24—31 weeks of gestation in
this population. These findings are consistent with previous randomized control trials and other recent studies
reporting that there is no significant difference in the incidence of NEC between placebo and antenatal MgSO₄
treatment groups8,12–20.

If magnesium causes intestinal injury, the pathogenesis for that injury is unclear. However, the biologic
effect of magnesium on intestinal muscle contractility may be a possible mechanism to cause gastrointestinal
complications. Magnesium ions can cross the placenta rapidly and its fetal levels increase in proportion with its
maternal levels31,22. Magnesium ions can replace calcium ions, disrupt actin and myosin interactions, and reduce
contractility, thus generating atony of intestine and fecal impaction23. They can also increase mesenteric arterial
resistance and reduce mesenteric blood flow24. The hypomotility of intestine is also due to cholinergic effect of
magnesium24. Hypomotility may lead to increased water absorption, the formation of stool plugs, and overgrowth
of bacteria. An intestinal plug can increase proximal intraluminal pressure which can be particularly detrimental
in setting of immature intestine along with decreased blood flow to the intestine of preterm neonates8. However,
the effect of antenatal MgSO₄ exposure on development of NEC is not fully understandable, especially in infants
who developed late-onset NEC.

Feeding issue, including enteral or parenteral feeding, breast milk or formula feeding, and feeding intoler-
ance, is another important factor for development of NEC in premature infants14. In our study, early-onset
NEC (within 14 days after birth) occurred in 14/35 (40.0%), and this incidence was similar to that of a large
population-based cohort of 16,669 infants with gestational age < 33 weeks of gestation26. The development of
these early-onset NEC might be associated with failure of or delayed breast milk feeding27. Cesarean section,
especially emergency cesarean section, is associated with reduced breast milk feeding success or delayed breast
milk feeding initiation28. In our study, cesarean section rate was over 70% and most of them are performed as
emergency surgery. However, although there were no changes in the feeding protocol during the entire study
period, we were not able to collect the individual feeding data because this study was a retrospective medical
record review. This is one of the main limitations of this study.

The development of NEC is associated with many other contributing factors25,29. Among them, the most
important risk factor is prematurity. The earlier the gestational age at delivery, the higher the risk of NEC25.
In our study, the incidences of NEC and SIP in the infants born at less than 26 weeks of gestation were higher
than those in the infants born at 26 weeks of gestation or more. Previous studies reporting the association of
antenatal MgSO₄ with NEC and/or SIP have included extremely preterm babies born at less than 25—26 weeks of
gestation14,15. Other studies reporting a lack of association between antenatal MgSO₄ treatment and NEC or SIP
have included preterm infants born at ≤ 28—34 weeks of gestation15–16. We included infants born at 24—31 weeks
of gestation, because the indication of antenatal MgSO₄ treatment protocol for fetal neuroprotection in our
institute was those who were at risk of preterm birth at 24—31 weeks of gestation, and the objective of this study
was to investigate whether these protocol changes might have any effect on the risk of NEC in this population.
Our results showed that antenatal MgSO₄ treatment per se or for fetal neuroprotection was not associated
with increased risk of NEC or SIP in babies born at less than 26 weeks of gestation, as well as in babies born at
26—31 weeks of gestation. However, our sample size was limited to analyze the association between antenatal
MgSO₄ treatment and NEC or SIP in extreme preterm neonates born at less than 26 weeks of gestation. Therefore,

| Table 4. Neonatal outcomes according to exposure to antenatal MgSO₄ treatment for fetal neuroprotection. MgSO₄, magnesium sulfate; aOR, adjusted odds ratio; CI, confidence interval; NEC, necrotizing enterocolitis; PTB, preterm birth; SIP, spontaneous intestinal perforation. 1 Adjusted for plurality, antenatal corticosteroids treatment, nifedipine treatment, and gestational age at delivery. |
|---|---|---|---|---|---|---|
| | MgSO₄ unexposed | MgSO₄ exposed for fetal neuroprotection | P value | OR (95% CI) | P value |
| NEC (2 stage 2b) | | | | | |
| All infants | 26/509 (5.1) | 6/126 (4.8) | 0.874 | 0.80 (0.31—2.03) | 0.636 |
| PTB < 26 weeks | 16/122 (13.1) | 2/32 (6.3) | 0.368 | 0.44 (0.09—2.09) | 0.299 |
| PTB 26—31 weeks | 10/387 (2.6) | 4/94 (4.3) | 0.490 | 1.41 (0.42—4.72) | 0.576 |
| NEC perforation | | | | | |
| All infants | 18/509 (3.5) | 3/126 (2.4) | 0.516 | 0.59 (0.17—2.06) | 0.405 |
| PTB < 26 weeks | 13/122 (10.7) | 1/32 (3.1) | 0.303 | 0.29 (0.04—2.33) | 0.242 |
| PTB 26—31 weeks | 5/387 (1.3) | 2/94 (2.1) | 0.627 | 1.41 (0.26—7.53) | 0.690 |
| SIP | | | | | |
| All infants | 9/509 (1.8) | 4/126 (3.2) | 0.318 | 1.84 (0.55—6.20) | 0.325 |
| PTB < 26 weeks | 4/122 (3.3) | 2/32 (6.3) | 0.605 | 1.88 (0.31—11.37) | 0.492 |
| PTB 26—31 weeks | 5/387 (1.3) | 2/94 (2.1) | 0.627 | 1.59 (0.30—8.52) | 0.586 |
The optimal antenatal MgSO4 regimen for fetal neuroprotection in terms of dose, maintenance dose, duration, timing, or repeat treatment has not been established yet. Different protocols of antenatal MgSO4 treatment might be another plausible cause of conflicting results about the effect of antenatal MgSO4 treatment on the risk of NEC. The antenatal MgSO4 treatment protocol for fetal neuroprotection in previous studies consisted of loading dose varying from 4 to 6 g intravenous over 20–30 min and maintenance dose varying from none to 1 g or 2 g per hour for 12 h or 24 h. In studies showing adverse neonatal gastrointestinal outcomes in the setting of antenatal MgSO4 exposure, higher loading dose (6 g) and higher maintenance dose (2 g per hour) were used than those in our study. Total dose of antenatal MgSO4 treatment for fetal neuroprotection was higher (50.9 ± 45.7 g or 33.0 ± 19.8 g) than that of our study (6.3 ± 10.3 g). However, studies that used lower dose of MgSO4 (4 g of loading dose only or 4 g of loading dose and 1 g per hour of maintenance dose for 24 h), including the Australasian collaborative trial (ACTOMgSO4), European Trial (PREMAG), and our current study, found no relationship between antenatal MgSO4 treatment and NEC or SIP. Therefore, it is necessary to establish optimum regimen for fetal neuroprotection to achieve maximal effectiveness with minimal adverse effects for both fetus and mother, especially for those at risk of preterm birth at less than 26 weeks of gestation.

The strength of our study is that it is a relatively large cohort study examining the association of antenatal MgSO4 treatment with risk of NEC in preterm neonates delivered from 24 + 0 weeks to 31 + 6 weeks. Our study investigated neonates with a large sample size of 756 in spite of a single center study like previous two studies. Whereas previous studies investigated neonates with a relatively small sample size to demonstrate the association of antenatal MgSO4 with NEC and/or SIP. However, our study may be underpowered because the sample size was not enough to show a difference in neonatal outcome, especially for the subgroup analyses stratified by gestational age at delivery. Besides, by comparing neonatal outcome before and after two major changes in the antenatal MgSO4 treatment protocol, we were able to analyze the effect of antenatal MgSO4 treatment on the risk of NEC. However, subjects in the three time period groups were not comparable regarding baseline characteristics and other predictor variables. Therefore, we performed multivariable analyses to adjust for potential confounding factors. In addition, there might be other unidentifiable confounding factors such as feeding issues and practice changes in obstetrics and neonatology over the study period that should have been controlled for the multivariable analysis.

This study was further limited by its inherent disadvantage of a retrospective study design including information bias. However, we tried to minimize information bias by blinding chart reviewers to study question and hypothesis. This study was also limited by inability to determine the total dose, timing, and retreatment of MgSO4 administration, especially in women who were transferred from other hospitals.

In conclusion, antenatal MgSO4 treatment for fetal neuroprotection was not associated with NEC, mortality, or other short-term neonatal outcomes in this population. A further study is needed to uncover the long-term neurodevelopmental outcome of these babies.

Methods
This was a retrospective cohort study of neonates delivered from 24 + 0 weeks to 31 + 6 weeks of gestation in Samsung Medical Center, Seoul, Korea from January 2012 to June 2018. Fetal death and major congenital anomalies were excluded. Medical records of mothers and babies were examined independently by obstetricians and neonatologists. Researchers who were blinded to maternal data including antenatal MgSO4 treatment reviewed the neonatal data for outcome. This study was approved by the Institutional Review Board for Clinical Research at Samsung Medical Center (IRB No 2019-04-051) and exemption for informed consent was granted because this was a retrospective chart review study. All methods were carried out in accordance with relevant guidelines and regulations.

Subjects were classified into three groups: period 1, from January 2012 to December 2013 when antenatal MgSO4 treatment for fetal neuroprotection was not adopted; period 2, from January 2014 to March 2016 when the treatment was routinely used; and period 3, from April 2016 to June 2018 when the treatment was withdrawn because of concern of increased risk of NEC.

Maternal demographic characteristics included maternal age at delivery, body mass index, parity, and plurality. Pregnancy outcomes reported included indications of antenatal MgSO4 treatment, type and cycle of antenatal corticosteroids therapy, type of tocolysis treatment, indications for delivery, mode of delivery, and histologic chorioamnionitis. Indications for delivery were classified into preterm labor, preterm premature rupture of membranes, and maternal–fetal indication such as preeclampsia, placenta previa, placenta abruption, and intrauterine growth restriction. Indications of antenatal MgSO4 treatment use were fetal neuroprotection, severe preeclampsia, and tocolysis.

MgSO4 treatment protocol for fetal neuroprotection consisted of intravenous loading dose of 4 g over 15 to 20 min followed by maintenance infusion of 1 g per hour for 24 h for women having imminent preterm birth within 24 h. Maintenance dose was discontinued if delivery did not occur within 24 h and delivery was no longer considered imminent. It was resumed if the risk of imminent delivery recurred within 6 h. Repeat loading dose and subsequent maintenance therapy were given if risk of imminent delivery recurred after 6 h. MgSO4 for treatment of severe preeclampsia and eclampsia was given with the same loading dose followed by a maintenance infusion of 1 g per hour and discontinued at 24 h after delivery. MgSO4 was not used for tocolysis in our institute. Women who were given MgSO4 for tocolysis in our study population were transferred from other hospitals. Therefore, the exact protocol or dose of MgSO4 used in these women was unknown.

Neonatal outcome measures included gestational age at delivery, sex, birth weight, Apgar scores, and neonatal mortality and morbidity. The primary outcome of our analysis was occurrence of NEC (≥ stage 2b). NEC was
diagnosed based on modified Bell's staging criteria\(^2\). Stage 2b was defined as mild metabolic acidosis (pH < 7.2), mild thrombocytopenia (platelet < 150,000/μL), absent bowel sounds, definite abdominal tenderness with or without abdominal cellulitis or right lower quadrant mass, and portal venous gas with or without ascites. Stage 3a was defined as systemic, intestinal, radiologic signs of stage 2b plus hypotension, bradycardia, disseminated intravascular coagulation, neutropenia, signs of generalized peritonitis, marked tenderness, and distention of abdomen, and definite ascites. Stage 3a was defined as systemic, intestinal, radiologic signs of stage 3a plus pneumoperitoneum. Early-onset NEC was defined as NEC diagnosed within 14 days after birth, and late-onset NEC was defined as NEC diagnosed more than 14 days after birth\(^2\). Spontaneous intestinal perforation (SIP) was defined as radiological evidence of perforation, a lack of clinical features of NEC, the absence of radiological features of intestinal ischemia (fixed dilated bowel loops, pneumatosis intestinalis), and/or an intra-operative surgical report and/or histopathology assessment indicating a perforation located in the ileum and on the anti-mesenteric border\(^3\). As a unit policy, minimal enteral feedings (20 mL/kg/day) were initiated within the first 3–4 h after birth and usually maintained for a week for infants with gestational age of less than 26 weeks. After that, feedings were gradually increased to reach full enteral feeding (150 mL/kg/day) according to the infant’s tolerance. For infants with gestational age of 26 weeks or greater, feedings were started within the first 3–4 h and advanced by 20 mL/kg/day until full enteral feedings. Infants were fed human breast milk; preterm formula milk was given in the absence of mother’s own milk. The management of feeding intolerance and the decision of continuing or discontinuing feeding were done by the attending neonatologists. There were no changes in the feeding protocol during the entire study period.

Obtained data were analyzed using the Statistical Package for Social Sciences version 24 (SPSS Statistics; IBM, Armonk, NY, USA). Maternal characteristics, pregnancy, and neonatal outcomes were compared across the three time periods. They were also compared between the infants exposed and unexposed to antenatal MgSO\(_4\) and between the infants exposed and unexposed to antenatal MgSO\(_4\) for fetal neuroprotection. Continuous variables were compared using independent-sample parametric (analysis of variance) or nonparametric (Kruskal–Wallis test) tests depending on data normality. Categorical variables were compared using Chi-square test or Fisher’s exact test when one or more expected value was less than 5. Bonferroni test was used for post-hoc analysis to correct for multiple comparisons. Multiple logistic regression analysis was performed to evaluate effects of potential confounding variables such as plurality, antenatal corticosteroids treatment, nifedipine treatment, and gestational age at delivery. Results were considered statistically significant when p value was less than 0.05. For multiple comparisons, \( p \) value was adjusted to 0.017 (0.05/3) by Bonferroni correction.

**Ethical approval.** This study was approved by the Institutional Review Board for Clinical Research at Samsung Medical Center at April 25, 2019 (IRB No 2019-04-051).

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**References**

1. Lazard, E. M. A preliminary report on the intravenous use of magnesium sulphate in puerperal eclampsia. *Am. J. Obstet. Gynecol.* **9**, 178–188 (1925).

2. Hunter, L. A. & Gibbins, K. J. Magnesium sulfate: Past, present, and future. *J. Midwif. Womens Health* **56**, 566–574. https://doi.org/10.1111/j.1542-1971.2011.01211.x (2011).

3. Mittendorf, R. Magnesium sulfate tocolysis: Time to quit. *Obstet. Gynecol.* **109**, 1204–1205. https://doi.org/10.1097/01.AOG.0000263775.75185.1e (2007).

4. Pryde, P. G. & Mittendorf, R. Contemporary usage of obstetric magnesium sulfate: Indication, contraindication, and relevance of dose. *Obstet Gynecol* **114**, 669–673. https://doi.org/10.1097/AOG.0b013e3181b4380e (2009).

5. Costantin, M. M., Weiner, S. J., Eunice Kennedy Shriver National Institute of Child Health & Human Development Maternal-Fetal Medicine Units Network. Effects of antenatal exposure to magnesium sulfate on neuroprotection and mortality in preterm infants: a meta-analysis. *Obstet Gynecol* **114**, 354–364. https://doi.org/10.1097/AOG.0b013e3181ae98e2 (2009).

6. Doyle, L. W., Crowther, C. A., Middleton, P., Marret, S. & Rouse, D. Magnesium sulphate for women at risk of preterm birth for neuroprotection of the fetus. *Cochrane Database Syst Rev*, CD004661. https://doi.org/10.1002/14651858.CD004661.pub3 (2009).

7. Conde-Agudelo, A. & Romero, R. Antenatal magnesium sulphate for the prevention of cerebral palsy in preterm infants less than 34 weeks’ gestation: A systematic review and metaanalysis. *Am. J. Obstet. Gynecol.* **200**, 595–609. https://doi.org/10.1016/j.amjog.2009.04.005 (2009).

8. Garg, B. D. Antenatal magnesium sulphate is beneficial or harmful in very preterm and extremely preterm neonates: A new insight. *J. Matern. Fetal Neonatal Med.* **32**, 2084–2090. https://doi.org/10.1080/14767058.2018.1424823 (2019).

9. Zeng, X., Xue, Y., Tian, Q., Sun, R. & An, R. Effects and safety of magnesium sulphate on neuroprotection of the fetus. *AJP Rep.* **6**, e148–e154. https://doi.org/10.55556/0036-1581059 (2016).

10. Conde-Agudelo, A., Romero, R. & Costantin, M. Magnesium sulphate for the prevention of cerebral palsy in preterm infants less than 34 weeks’ gestation: A meta-analysis based on PRISMA guidelines. *Medicine (Baltimore)* **95**, e2451. https://doi.org/10.1097/MD.0000000000002451 (2016).

11. American College of Obstetricians, Gynecologists Committee on Obstetric Practice & Society for Maternal-Fetal Medicine. Committee Opinion No. 455: Magnesium sulfate before anticipated preterm birth for neuroprotection. *Obstet. Gynecol.* **115**, 669–671, http://doi.org/10.1097/AOG.0b013e3181d1ffaf (2010).

12. Rattray, B. N. et al. Antenatal magnesium sulphate and spontaneous intestinal perforation in infants less than 25 weeks gestation. *J. Perinatol.* **34**, 819–822. https://doi.org/10.1097/PPJ.0b013e318143f4af (2014).

13. Kamyar, M., Clark, E. A., Yoder, B. A., Varner, M. W. & Manuck, T. A. Antenatal magnesium sulphate, necrotizing enterocolitis, and death among neonates < 28 weeks gestation. *AJP Rep.* **6**, e48–e54. https://doi.org/10.55556/0036-1581059 (2016).

14. Downey, L. C. et al. Association of in utero magnesium exposure and spontaneous intestinal perforations in extremely low birth weight infants. *J. Perinatol.* **37**, 641–644. https://doi.org/10.1016/j.jperi.2016.06.024 (2017).

15. Shalabi, M. et al. Antenatal exposure to magnesium sulphate and spontaneous intestinal perforation and necrotizing enterocolitis in extremely preterm neonates. *Am. J. Perinatol.* **34**, 1227–1233. https://doi.org/10.1055/s-0037-1603344 (2017).
15. Edwards, J. M., Edwards, L. E., Swamy, G. K. & Grotegut, C. A. Effect of cord blood magnesium level at birth on non-neurologic neonatal outcomes. Am. J. Perinatol. 36, 3–7. https://doi.org/10.1055/s-0038-1627907 (2019).

16. Mikhail, M. et al. Lack of evidence for time or dose relationship between antenatal magnesium sulfate and intestinal injury in extremely preterm neonates. Neonatology 115, 371–378. https://doi.org/10.1159/000497412 (2019).

17. Rouse, D. J. et al. A randomized, controlled trial of magnesium sulfate for the prevention of cerebral palsy. N. Engl. J. Med. 359, 495–505. https://doi.org/10.1056/NEJMoa0801187 (2008).

18. Crowther, C. A., Hiller, J. E., Doyle, L. W., Haslam, R. R. & Australasian Collaborative Trial of Magnesium Sulphate Collaborative Group. Effect of magnesium sulfate given for neuroprotection before preterm birth: a randomized controlled trial. JAMA 290, 2669–2676 (2003).

19. Marret, S. et al. Effect of magnesium sulphate on mortality and neurologic morbidity of the very-preterm newborn (of less than 33 weeks) with two-year neurological outcome: results of the prospective PREMAG trial. Gynecol. Obstet. Fertil. 36, 278–288. https://doi.org/10.1016/j.gyobfte.2008.01.012 (2008).

20. Shepherd, E. et al. Antenatal magnesium sulphate and adverse neonatal outcomes: A systematic review and meta-analysis. PLoS Med. 16, e1002988. https://doi.org/10.1371/journal.pmed.1002988 (2019).

21. Cruikshank, D. P., Pitkin, R. M., Reynolds, W. A., Williams, G. A. & Hargis, K. G. Effects of magnesium sulfate treatment on perinatal calcium metabolism. J. Maternal and fetal responses. Am. J. Obstet Gynecol. 134, 243–249 (1979).

22. Aikawa, J. K. & Bruns, P. D. Placental transfer and fetal tissue uptake of Mg28 in the rabbit. Proc. Soc. Exp. Biol. Med. 105, 95–98 (1960).

23. Lu, J. F. & Nightingale, C. H. Magnesium sulfate in eclampsia and pre-eclampsia: Pharmacokinetic principles. Clin. Pharmacokinet. 38, 305–314. https://doi.org/10.2165/00003088-20003804-00002 (2000).

24. Havranek, T., Ashmeade, T. L., Afanador, M. & Carver, J. D. Effects of maternal magnesium sulfate administration on intestinal blood flow velocity in preterm neonates. Neonatology 100, 44–49. https://doi.org/10.1159/000190499 (2011).

25. Gephart, S. M., McGrath, J. M., Effken, J. A. & Halpern, M. D. Necrotizing enterocolitis risk: state of the science. Adv. Neonatal Care 12, 77–87; quiz 88–79. https://doi.org/10.1097/ANC.0b013e31824ce94 (2012).

26. Yee, W. H. et al. Incidence and timing of presentation of necrotizing enterocolitis in preterm infants. Pediatrics 129, e298-304. https://doi.org/10.1542/peds.2011-2222 (2012).

27. Sullivan, S. et al. An exclusively human milk-based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk-based products. J. Pediatr. 156, 562–567 e561. https://doi.org/10.1016/j.jpeds.2009.10.040 (2010).

28. Hobs, A. J., Mannion, C. A., McDonald, S. W., Brockway, M. & Tough, S. C. The impact of caesarean section on breastfeeding initiation, duration and difficulties in the first four months postpartum. BMC Pregnancy Childbirth 16, 90. https://doi.org/10.1186/1471-2393-16-90 (2016).

29. Lee, J. S. & Polin, R. A. Treatment and prevention of necrotizing enterocolitis. Semin. Neonatol. 8, 449–459. https://doi.org/10.1016/S0891-1798(03)00123-4 (2003).

30. Bain, E., Middleton, P. & Crowther, C. A. Different magnesium sulphate regimens for neuroprotection of the fetus for women at risk of preterm birth. Cochrane Database Syst. Rev., CD0039302, https://doi.org/10.1002/14651858.CD0039302.pub2 (2012).

31. Marret, S. et al. Magnesium sulphate given before very-preterm birth to protect infant brain: The randomised controlled PREMAG trial*. BJOG 114, 310–318. https://doi.org/10.1111/j.1471-0528.2006.01162.x (2007).

32. Shah, J. et al. Intestinal perforation in very preterm neonates: Risk factors and outcomes. J. Perinatol. 35, 595–600. https://doi.org/10.1038/jp.2015.41 (2015).

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S.O., C.-R.R., Y.S.C., W.S.P. and S.-J.C. conceived the study, Y.-S.C. and S.-J.C. designed the study, J.Y.H., Y.-S.C., Y.-M.K., J.-H.S., H.S.K., S.I.S., and S.Y.A. performed the study, J.Y.H. and S.-J.C. analyzed the data, and J.Y.H. and S.-J.C. wrote the main manuscript text. All authors reviewed the manuscript.

Competing interests
The authors declare no competing interests.

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