Magnesium sulfate versus esomeprazole impact on the neonates of preeclamptic rats

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Abstract

Objective: Preeclampsia represents a major complication of pregnancy, associated with greater maternal and fetal complications. We compared the effects of esomeprazole (a proton pump inhibitor) and magnesium sulfate (MgSO4) on the deleterious effects observed on the mother and neonates in experimentally induced preeclampsia in rats.

Study design: Preeclampsia was induced in pregnant rats with NG-nitro-L-arginine methyl ester (L-NAME) starting from day 10-till end of pregnancy. Pregnant rats were divided into four groups: control pregnant; untreated preeclampsia; preeclamptic rats treated with MgSO4 and preeclamptic treated with esomeprazole. Treatment was started on day 14 and continued until end of pregnancy. Systolic blood pressure, gestation duration, the total number of pups/fetal resorption, pups birth weight, and histopathology examination of the pup’s organs were recorded.

Results: In comparison with the L-NAME group, the MgSO4 and esomeprazole treatment reduced the values of systolic blood pressure. MgSO4 normalized gestational duration while esomeprazole prolonged it (post-term pregnancy); both restored number of delivered pups; with no statistical differences between the numbers of died pups between the four groups studied while with esomeprazole, out of 10 pregnant females, 2 of them had complete intrauterine fetal resorption; esomeprazole normalized birth weight and histological structure of fetal liver, kidney, and brain. On the other side, MgSO4 treatment gave rise to lower than normal birth weight and minimal tissue damage.

Conclusion: Esomeprazole and MgSO4 improved systolic blood pressure, prevented preterm labor and restored numbers of pups delivered and fetal weight. Esomeprazole prolonged gestational period post-term with subsequent improving reproductive outcome.

Introduction

One of the most serious complications of pregnancy is Preeclampsia (PE). Fetal complications are linked to the severity of PE. Clinicians are often forced to deliver early preeclampsia mother to prevent major maternal morbidity, but in doing so, inflict severe prematurity on the fetus. The main impact on the fetus is low nutrition as a result of uteroplacental vascular insufficiency, which leads to growth restriction. Because oxidative stress and free radicals may play roles in several neonatal diseases, a direct effect of preeclampsia on the neonatal outcome is expected [1].

Magnesium may influence blood pressure by modulating vascular tone and structure, through its effects on various biochemical reactions that control vascular contraction/dilation, growth/apoptosis, differentiation, and inflammation. Magnesium may be physiologically important in blood pressure regulation whereas changes in magnesium levels could contribute to the etiology of hypertension [2].

Although Magnesium sulfate (MgSO4) is one of the main lines of treatment for preeclampsia, and is often used to prevent the progression from preeclampsia to eclampsia, it carries risk of fetal abnormalities as it is AU TGA and US FDA pregnancy category: D [3,4]. The American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine have long supported the short-term use of MgSO4 in obstetric care for appropriate conditions and durations of treatment [4].

The preeclamptic placenta releases antiangiogenic soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) into the maternal circulation. These factors cause widespread maternal endothelial dysfunction and organ injury [5].

Esomeprazole is a proton pump inhibitor, US FDA pregnancy category B, widely used to treat women with gastric reflux in...
pregnancy. Esomeprazole decreases secretion of sFlt-1 and sEng from placenta and endothelial cells, also has antioxidant properties and strongly minimizing endothelial dysfunction [6].

If a reasonable and safe treatment was available that could hinder the progression of preeclampsia, clinicians could safely delay delivery and improve fetal outcome.

The purpose of this study was to compare MgSO4 and esomeprazole administered from gestational day 14 until the end of pregnancy, on the improvements in blood pressure, gestational duration and fetal outcome of preeclamptic rats.

Methods

Animals and ethics statement

Adult females Sprague Dawley albino rats, 14–16 weeks old, weighing 180–200 g were used in this study. They were housed in standard laboratory conditions under a 12 h light/dark cycle and controlled temperature of 22 ± 3 °C, with free access to food and water. The study was approved by Cairo University, The Institutional Animal Care and Use Committee (CU-IACUC) with the approval number “CU/III/S/6/16”.

Female rat’s conception

Prior to the experiment, the rats were acclimated to laboratory conditions for 7 days. Females were placed with males overnight and examined the next morning for the presence of sperm in vaginal smears. Day 0 of pregnancy was defined as the day when spermatozoa were found in a vaginal smear [7].

Induction of PE and experimental design

Pregnant rats were treated from day 10 to the end of pregnancy with L-NAME (L-NAME powder, Elnasr Pharmaceutical Co, Egypt, dissolved in DW) 80 mg/kg/day by oral gavage [8]. Preeclampsia onset was evident by a significant increase in blood pressure [9]. Rats were divided into four groups (ten rats per group): control pregnant rats; untreated preeclampsia; preeclamptic rats treated with MgSO4 (Magnesium sulfate powder, Hijma Egypt, dissolved in DW) 500 mg/kg/day [10] by oral gavage and preeclamptic rats treated with esomeprazole (Esomeprazole powder, Glaxo Wellcome Egypt, dissolved in polyethylene glycol 400) 3.5 mg/kg/day [11] by oral gavage. Treatment was started on day 14 and continued until end of pregnancy.

Measurements

Blood pressure measurement

The systolic blood pressure (SBP) of each rat in different groups was measured 5 times; at the start of the experiment, at the gestational days 9, 14, 17 and at the end of the experiment. The SBP was measured non-invasively using a rat tail sphygmomanometer (Panlab, USA). The SBP for each rat was calculated as the mean of at least 3 readings.

Gestational duration

For every pregnant female rat in each group, gestational duration was estimated in days starting from gestational day 0 (day of detection of sperms in vaginal smear) till the end of pregnancy.

Total number of pups/Fetal resorption

After delivery, the total number of pups (living and died) and fetal resorption for each pregnant female rat in each group was estimated.

Pups birth weight

After delivery, pups birth weight was estimated in gram.

Histopathology examination of the pup’s organs

Pups were euthanized and their liver, kidneys, and brain tissue specimens were fixed in 10% formol saline, then trimmed off, washed and dehydrated in ascending grades of alcohol. The dehydrated specimens were then cleared in xylene, embedded in paraffin blocks and sectioned at 4–6 μm thick. The obtained tissue sections were deparaffinized using xylol and stained using hematoxylin and eosin (H&E) for histopathological examination through the electric light microscope [12].

Statistical analysis

Data were coded and entered using the statistical package SPSS version 24. Data were summarized using mean and standard error of the mean (SEM) in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data.

Comparisons between groups were done using ANOVA with post hoc test in normally distributed quantitative variables while non-parametric Kruskal-Wallis test and Mann-Whitney test were used for non-normally distributed quantitative variables [13]. For comparison of serial measurements within each group repeated measures ANOVA was used in normally distributed quantitative variables while non-parametric Friedman test was used for the non-normally distributed quantitative variable [14]. For comparing categorical data, Chi-square (χ2) test was performed. An exact test was used instead when the expected frequency is less than 5 [15]. P-values less than 0.05 were considered as statistically significant.

Results

Gestational duration

Gestational duration was decreased significantly in preeclamptic untreated rats compared to the other groups; treated with

<table>
<thead>
<tr>
<th>Groups Studied</th>
<th>Gestational duration (mean ± SEM//days)</th>
<th>Control pregnant</th>
<th>Untreated preeclampsia</th>
<th>Preeclampsia+ MgSO4</th>
<th>Preeclampsia+ Esomeprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>21.20 ± 0.20</td>
<td>16.60 ± 0.16</td>
<td>21.10 ± 0.31</td>
<td>24.30 ± 0.86</td>
</tr>
</tbody>
</table>

* p < 0.05: significant compared to control pregnant rats.

* p < 0.05: significant increase compared to untreated preeclampsia group.

** p < 0.05: significant increase compared to preeclampsia group treated with MgSO4.

Table 1

Gestational duration measured in days for every pregnant female rat (n = 10) in each group starting from gestational day 0 till the end of pregnancy.

* p < 0.05: significant increase compared to untreated preeclampsia group.

** p < 0.05: significant increase compared to preeclampsia group treated with MgSO4.
MgSO4 showed normalization of gestational duration; while preeclamptic rats treated with esomeprazole showed a significant increase in gestational duration (post-term pregnancy) (Table 1).

**Systolic blood pressure**

In response to L-NAME, SBP started to rise significantly on gestational day 14 (4 days after induction with L-NAME) in preeclamptic groups till the end of pregnancy (Graph 1). There was significant decrease (p < 0.05) in SBP values in both preeclamptic groups treated with MgSO4 and those treated with esomeprazole with an insignificant difference.

### Total number of pups/Fetal resorption

Preeclamptic untreated rats delivered a lower number of pups; while those treated with MgSO4 and esomeprazole showed a statistically significant increase in the total number of pups with no significant difference in between and to control (Table 2).

The number of died pups was nearly the same between the four groups studied (Table 2).

Out of 10 pregnant females in preeclamptic group treated with esomeprazole, 2 of them had complete intrauterine fetal resorption.

### Pups birth weight

Pups birth weight of untreated preeclamptic rats and those treated with MgSO4 was decreased significantly; While birth weights of pups delivered from preeclamptic rats treated with esomeprazole were significantly increased compared to untreated preeclampsia and preeclamptic rats treated with MgSO4 (Table 3).

### Histopathology examination of the pups

#### Pups from control pregnant mothers

Light microscopic examination of neonatal livers of control pregnant mother showed ill-defined demarcation of hepatic lobules and the inter-lobular connective tissue was poorly developed. In each hepatic lobule, hepatocytes were arranged as irregular, branching and interconnected cords originating from a central vein and go peripherally. Blood sinusoids were seen in-between the hepatic cords. The quantity of hepatocytes appeared greater than hepatoblasts which have large nucleus basophilic with little cytoplasm. The liver also had hematopoietic function characterized by the presence of hematopoietic progenitor cells and megakaryocyte lineage cells. Erythroblasts and Kupffer cells within sinusoids also were also seen (Fig. 1a).

The kidneys were bilaterally present and normal in shape in all pups of this group. On histological examination, the sagittal section of kidneys showed well developed cortical renal corpuscles; these contained loops of glomerular capillaries, surrounded by Bowman’s capsule, having visceral and parietal layers and lined by simple squamous epithelium, with capsular space in-between. The cortical region also showed cross-section of proximal and distal convoluted tubules lined by simple cuboidal epithelium. All these structures were embedded in the interstitium (Fig. 1b).

Brain tissue section of pups of the control group showed an incomplete development of brain architecture. The neuronal cells appeared clear with a prominent basophilic nucleus (Fig. 1c).

#### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Groups Studied</th>
<th>Untreated preeclampsia</th>
<th>Preeclampsia + MgSO4</th>
<th>Preeclampsia + Esomeprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control pregnant</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total number of pups</td>
<td>8.30 ± 0.56</td>
<td>4.40 ± 0.43</td>
<td>7.44 ± 0.29</td>
<td>7.71 ± 0.57**</td>
</tr>
<tr>
<td>Number of died pups</td>
<td>0</td>
<td>0.40 ± 0.22</td>
<td>0.80 ± 0.70</td>
<td>0</td>
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<tr>
<td>Intrauterine resorption</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

*p < 0.05: significant decrease compared to control pregnant rats
**p < 0.05: significant increased compared to untreated preeclampsia

**Graph 1.** Mean Systolic Blood Pressure (mmHg, ANOVA) measurements in all groups throughout gestational period (n = 10).

*p < 0.05: significant increase compared to control pregnant rats
#p < 0.05: significant decrease compared to untreated preeclampsia group
The visceral offspring nucleus, giosis during Pups cal tubules showed blasts and capillaries and debris. Fig. 1. Tissue pathology from pups of control mother (H&Ex200).

Fig. 1. Tissue pathology from pups of control mother (H&Ex200).

a. Hepatic tissue section showing hepatic cords and sinusoids contained erythroblasts and Kupffer cells.
b. Sagittal section of kidney showing a part of the cortical region containing glomerulus within Bowman’s capsule and renal tubules.
c. Brain tissue showing neuronal cells appeared clear with prominent basophilic nucleus.

histological findings in this group were recorded in all full-term rat offspring and complete development of brain tissue occurred during the first two weeks of life.

Pups from untreated preeclamptic mother
Neonatal liver delivered from untreated preeclamptic mother showed ill developed hepatic lobules with distorted hepatic cords. The hepatocytes were bloated with large, spherical droplets of fat in a focal and/or diffuse manner. The quantity of hepatoblasts appeared greater than hepatocytes. Apoptosis of hepatic cells with complete depletion of hematopoietic progenitor cells (Fig. 2a).

Kidneys appeared oedematous, pale and doughy. On histological examination, the sagittal section of kidney revealed ill developed renal corpuscles that is composed of few numbers of capillaries loops and surrounded by Bowman’s capsule. The renal tubules showed coagulative necrosis of its epithelial lining with intact basement membrane (Fig. 2b).

Histological section of brain tissue specimens showed spongiosis consisted of irregular clear spaces in both gray and white matter. Neurons were shrunken and darkly stained with a small nucleus, with a significant reduction in cell density (Fig. 2c).

Pups of preeclamptic mother treated with MgSO4
The structure of hepatic parenchyma appeared resembling to control group. The quantity of hepatocytes greater than hepatoblasts was seen. Blood spaces contained hematopoietic progenitor cells and Kupffer cells were also noticed. The hepatic lobules showed a fewer number of apoptotic bodies in comparison with untreated and treated by esomeprazole groups (Fig. 3a).

Kidneys of this animal group showed a slight increase in size. The sagittal tissue section showed glomeruli with partial and visceral layers with ill-developed glomerular tufts. The renal tubules lined by simple cuboidal epithelium. Some tubules showed damage to its basement membrane with intra-tubular cellular debris (Fig. 3b).

Brain tissue section appeared resembling control group without spongiosis and reduction of neuronal cells (Fig. 3c).

Pups of preeclamptic mother treated with esomeprazole
The hepatic parenchyma showed many lobules with disorganization of hepatic cords with irregular blood spaces contained hematopoietic progenitor cells and Kupffer cells. Few hepatoblasts and apoptosis of few hepatocytes also were seen (Fig. 4a).

The pathological finding of renal tissue of this group revealed similar alterations seen in Pups of preeclamptic mother treated with MgSO4 (Fig. 4b).

Brain tissue section showed mild spongiosis in compared with untreated group. Also, the neuronal cells population like control group (Fig. 4c).

Discussion
The current curative treatment for preeclampsia is delivery of the placenta as this removes the source of placental-derived factors responsible for the maternal organ injury. In preterm preeclampsia, clinicians are often forced to deliver the fetus preterm to save the mother [16]. A treatment that quenches the severity of the maternal disease could allow these pregnancies to safely advance to a gestation where neonatal outcomes are significantly improved.

The present study reveals that L-NAME administration resulted in the increase in SBP, preterm labor, deleterious effect on the total number of pups and their fetal birth weight. Placental ischemia and hypo-perfusion were reflected in pups’ kidneys, hepatocytes and brain tissues.

Treatment with MgSO4 decreased SBP, prevented preterm labor, and avoided the deleterious effects on the total number of pups and fetal birth weight. Relaxation of the arterial blood vessel and the increase of placental blood flow were reflected on fetal development which appeared histologically as increase in quantity of hepatocytes than hepatoblasts and blood spaces that contained

<table>
<thead>
<tr>
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<th>Preeclampsia + Esomeprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pups Weight (mean ±SEM/gm)</td>
<td>3.23 ± 0.03</td>
<td>1.54 ± 0.06</td>
<td>2.78 ± 0.05</td>
<td>3.15 ± 0.03</td>
</tr>
</tbody>
</table>

* p < 0.05: significant decrease compared to control pregnant.
** p < 0.05: significant increase compared to untreated preeclampsia rats.
*** p < 0.05: significant increase compared to preeclampsia rats treated with MgSO4.
Fig. 2. Tissue pathology from pups of Untreated Pre-eclamptic mother (H&Ex200).

a. Hepatic tissue section showing vacuolation and apoptosis of hepatocytes.
b. Sagittal section of kidney showing ill developed renal corpuscles and necrosis of renal tubules.
c. Brain tissue showing spongiosis consisted of irregular clear spaces.

Fig. 3. Tissue pathology from pups of Pre-eclamptic mother treated with MgSO4 (H&Ex200).

a. Hepatic tissue section showing few number of apoptotic bodies.
b. Sagittal section of kidney showing a part of the cortical region containing glomerulus within Bowman’s capsule and renal tubules.
c. Brain tissue showing neuronal cells appeared clear with prominent basophilic nucleus.

Fig. 4. Tissue pathology from pups of Pre-eclamptic mother treated with esomeprazole (H&Ex200).

a. Hepatic tissue section showing disorganized hepatic cords with irregular blood spaces contained hematopoietic progenitor cells.
b. Sagittal section of kidney showing glomeruli with partial and visceral layers with ill developed glomerular tufts.
c. Brain tissue showing mild spongiosis.
active hematopoietic progenitor cells. Brain tissue section appeared resembling control normal group without evidence of any damage.

The disturbance in proangiogenic and antiangiogenic factors is reported in preeclamptic pregnancies and is considered the main cause of the endothelial dysfunction. Placental ischemia and hypoperfusion decrease placental growth factor (PIGF) and produce high concentrations of soluble vascular endothelial growth factor receptor-1 (sVEGFR-1), and the transmembrane glycoprotein endoglin (ENG) [17]. Increased VEGF augments nitric oxide (NO) production that was found to have an important role in the mitogenic effect of VEGF during angiogenesis.

Nitric oxide and endothelin-1 are important endothelium derived mediators of vascular tone. NO system may play a role in the regulation of myometrial contractility, and that inappropriate synthesis of this mediator may trigger preterm delivery. Notably, the contractility of pregnant rat uterus was inhibited by NO and its precursor L-arginine [18]. NO generation would be up-regulated in myometrium and down-regulated in the cervix, assuring uterine relaxation and cervical competence, the opposite effects would occur during labor leading to myometrial contraction and cervical ripening. L-NAME treatment during pregnancy in rats causes eNOS inhibition that led to the reduction of NO which participates in the cardiovascular adaptation that occurs in a normal pregnancy [19], so vasoconstriction occurs and subsequent hypertension developed, also early myometrial contraction and cervical ripening with subsequent preterm labor [20].

There are many short- and long-term consequences of preeclampsia to the offspring [21]; fetal weight as well as its weight, is highly compromised, leading to various degrees of fetal morbidity, and fetal damage may be such as to cause fetal death. In later life, babies with intrauterine growth restriction (IGR) are more likely to develop chronic diseases.

Magnesium is a unique calcium antagonist as it can act on most types of calcium channels in vascular smooth muscle by inhibiting Ca2+ influx leading to inactivation of calmodulin-dependent myosin light chain kinase activity and decreased contraction [22,23]. Magnesium may act by stimulating the production of prostacyclin by endothelial cells causing vasodilation, or by inhibiting platelet aggregation. In patients with pregnancy-induced hypertension [22], MgSO4 treatment significantly decreased circulating levels of the angiotensin-converting enzyme. These actions may attenuate the endothelial dysfunction associated with preeclampsia. The endothelium appears to potentiate the vasorelaxant effects of magnesium through the nitric oxide–cyclic GMP and cyclooxygenase systems and prevent preterm labor [24].

In 2008, Rouse and colleagues [25] randomized MgSO4 infusions or placebo in 2241 women delivered between 24 and 31 weeks' gestation in a study of the effects of MgSO4 on perinatal death and/or cerebral palsy at 2 years of age. MgSO4 therapy was not found to be associated with perinatal death, neonatal hypotonia, or any other neonatal morbidity. Such therapy, however, was associated with a significant reduction in cerebral palsy, especially in infants born between 24 and 27 weeks' gestation. Esomeprazole significantly improved SBP, prolonged the gestational duration, prevented the deleterious effect on the total number of pups, fetal birth weight. The histological structure of fetal vital organs appeared in normal architecture without any significant pathological alterations.

Onda et al. [6] induced mouse model of preeclampsia and studied the effect of esomeprazole that was completely abrogated an increase in blood pressure. Esomeprazole potently vasodilated whole maternal blood vessels and reduced blood pressure in an animal model of preeclampsia [26].

Like sFlt-1, sENG also antagonizes endothelial receptor signaling by competing with full-length endoglin, decreasing its ability to bind with its co-receptors to maintain vessel homeostasis, causing widespread maternal endothelial dysfunction and organ injury. Preeclampsia is also associated with oxidative stress. Esomeprazole, a proton pump inhibitor decreases secretion of sFlt-1 and sENG from primary trophoblast, placental explants from normal and preeclamptic pregnancies, and from 2 types of primary endothelial cells. Esomeprazole vasodilates human vessels from normal and preeclamptic pregnancies and reduces blood pressure [6]. The ability of esomeprazole to dilate vessels and reduce blood pressure may be mediated through the endothelium, perhaps by increasing p-eNOS expression. PPIs seem to block endothelial dysfunction in vitro assays and upregulate endogenous antioxidant molecules and decrease secretion of cytokines from placental tissues and endothelial cells [6].

Esomeprazole showed significant improvement in reproductive outcome through reduction in sEng levels. Maynard et al. [27] explained that serum sEng may be a cause of the low-birth-weight babies in multiple gestation pregnancies.

Esomeprazol has the advantages that it is available in oral form and is pregnancy category B. If esomeprazole is proven to be effective at prolonging gestation in early onset preeclampsia with improvement in reproductive outcome, it would be the first treatment option for this group of vulnerable mothers and could play an important role in decreasing the unfavourable outcomes.

**Conclusion**

Both MgSO4 and esomeprazole prevented deleterious effects of preeclampsia on both maternal and fetal outcome. Superior efficacy was noted with esomeprazole in prolongation of gestational duration and increase in fetal birth weight. Further experimental and clinical studies are recommended to evaluate the efficacy of esomeprazole in the reduction of neonatal complications resulting from preeclampsia and prevention of eclampsia.

**References**
