Effect of oral magnesium supplementation on experimental pre-eclampsia induced by prolonged blockade of nitric oxide synthesis in pregnant rats

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Nitric oxide inhibitor L-NAME when given alone caused a significant rise in both systolic and diastolic pressure, an increase in 24 hr urinary protein excretion and reduction in weight of the litter as compared to control group. Supplementation of MgSO4 at lower dose (250mg/kg) did not inhibit this pre-eclamptic effect of L-NAME; but in higher doses (500 and 750mg/kg), it inhibited the pre-eclamptic action of L-NAME. The results suggest that administration of MgSO4 improves the foetal outcome and significantly prevents the development of symptoms of pre-eclampsia like hypertension and proteinuria.

Pre-eclampsia (PE) is a hypertensive disorder of pregnancy characterized by vasospasm, proteinuria and oedema. PE is an important cause of maternal and fetal morbidity and mortality. Nitric oxide (NO) synthesized by vascular endothelium plays a crucial role in the control and modulation of vascular tone during pregnancy. A reduced NO synthesis linked to a decreased NO synthase expression or activity has been reported in pre-eclamptic patients. Although magnesium sulphate (MgSO4) is now an established drug for the treatment of eclampsia and pre-eclampsia, its mechanism of action in this condition is still unclear. The most accepted mechanism is the calcium antagonist action of Mg at membrane Ca2+ channels or at intracellular sites. There is evidence which suggests that hypomagnesemia inhibits NO release from coronary endothelium. Therefore it can be hypothesized that magnesium ion has a role in the synthesis of NO. The present study has been undertaken in order to test this hypothesis and investigate whether Mg supplementation in rat model of pre-eclampsia would be able a) to prevent the increase in blood pressure; and b) to normalize foetal weight.

The study was conducted in virgin female wistar strain albino rats weighing between 150-200 g. They were housed in the departmental animal house at 65±2% RH, 23±2°C and 12:12 hr L:D cycle. They were fed on standard pellet chow and tap water ad libitum. The animals were acclimatized in the laboratory conditions for at least one week before experimentation.

After mating, the day of conception was determined by presence of spermatozoa in the vaginal lavage. The animals were divided into following 3 groups of 10 animals each:

Gr. A (control group): The animals were given vehicle (saline) starting on day 13-14 of gestation for 7 days.

Gr. B (L-NAME treated group): The pregnant rats were given nitric oxide synthase inhibitor L-NAME (50 mg/kg/day; ip) starting on day 13-14 of gestation for 7 days. This dose of L-NAME was chosen on the basis of previous study by Richer et al.

Gr. C (MgSO4 + L-NAME treated group): The pregnant rats were given nitric oxide synthase inhibitor L-NAME (50 mg/kg/day; ip) and MgSO4 (250, 500 and 750 mg/kg/day; po) starting on day 13-14 of gestation for 7 days. The doses of MgSO4 were chosen after conducting pilot study.

All the animals were weighed daily and systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in the conscious state using the tail cuff method (B.P.recorder 8006, Ugo Basile) as described by Bunag and Butterfield.

Animals were kept in metabolic cages from day 1 of pregnancy and 24 hr urine was collected till delivery for estimation of 24 hr urinary proteins using the Biuret reaction.

Duration of gestation was noted for each animal. After delivery, pups were removed, numbered and immediately weighed individually.

Statistical analysis—All the data are expressed as mean ± SE. Comparisons of mean values were carried out by ANOVA followed by Scheffe’s test.
Evaluation of maternal parameters during pregnancy—From day 14 of gestation till parturition maternal body weight was not significantly different in any of the experimental groups (Table 1).

Both SBP and DBP were significantly higher ($P < 0.05$) in the group B as compared to group A. Group C animals treated with MgSO$_4$ (250 mg/kg) alongwith L-NAME also produced similar rise in SBP and DBP. The B.P. started rising on day 14 and continued till delivery in both groups. In group C animals treated with higher doses of MgSO$_4$ (500 and 750 mg/kg) alongwith L-NAME, did not produce any rise in B.P. compared to group A animals (Fig. 1a and b).

Foetal effects—Gestation duration as compared to group A animals was not modified by any of the treatments. The litter weight was significantly reduced in group B animals. In group C, all the three doses of MgSO$_4$ when given with L-NAME did not produce a significant change in litter weight as compared to group A (Table 1).

Urinary proteins—Twenty-four hour urinary protein was significantly higher in the groups treated with L-NAME alone and L-NAME alongwith MgSO$_4$ (250 mg/kg) compared to control group A. In the groups treated with higher doses of MgSO$_4$ (500 and 750 mg/kg) there was no significant difference in the urinary proteins as compared to control group (Fig. 2 and Table 1).

The rat experimental pre-eclampsia model used in this study consisted of daily oral administration of L-NAME (50 mg/kg) to pregnant rats starting on day 13-14 of gestation. The procedure mimics in pregnant rats the classical signs of pre-eclamptic syndrome i.e. a progressive increase in B.P. and increase in urinary proteins$^{14,15}$. The reduction in litter weight may be due to relative ischaemia of the placenta due to reduction in the foetoplacental blood flow because of vasoconstriction$^{16}$.

Therefore, chronic inhibition of NO synthesis in gravid rats provides a simple animal model for pre-eclampsia as already demonstrated by other workers$^{16,17}$. Administration of L-arginine (NO precursor) effectively inhibits the action of L-NAME on blood pressure and heart rate in pregnant and nonpregnant rats$^{18}$. In this context it was thought worth investigat-

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### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>L-NAME</th>
<th>L-NAME + MgSO$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>109 ± 2</td>
<td>142 ± 2*</td>
<td>131 ± 2</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>83 ± 3</td>
<td>106 ± 3*</td>
<td>103 ± 3</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>151 ± 2</td>
<td>167 ± 3</td>
<td>160 ± 1</td>
</tr>
<tr>
<td>Litter number (n)</td>
<td>9.7 ± 1.5</td>
<td>9.3 ± 2.3</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Foetal weight (g)</td>
<td>5.3 ± 0.13</td>
<td>4.1 ± 0.13*</td>
<td>5.9 ± 0.1</td>
</tr>
<tr>
<td>Urinary proteins (mg/l)</td>
<td>8.8 ± 1.2</td>
<td>13.3 ± 0.5**</td>
<td>14.0 ± 0.9**</td>
</tr>
</tbody>
</table>

$P$ values: *$< 0.05$; **$< 0.01$; ***$< 0.001$.
The role of nitric oxide in the pathogenesis of pre-eclampsia in pregnant rats. Intracellular Mg\(^{2+}\) may be due to increased synthesis of NO or it could be due to general free-radical scavenging activity of Mg\(^{2+}\). It has also been shown that there is reduction in placental nitric oxide synthase activity in pre-eclampsia. However, the links of nitric oxide synthase activity and Mg\(^{2+}\) remain largely unexplored.

In conclusion, in a chronic NO deprivation-induced model of pre-eclampsia in rats, administration of Mg\(\text{SO}_4\) improves the foetal outcome and significantly prevents the development of symptoms of pre-eclampsia like hypertension and proteinuria.

References