

Different Effects of Pravastatin on Preeclampsia-like Symptoms in Different Mouse Models

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Abstract

Background: Pravastatin (Pra) exerts protective effects on preeclampsia. Preeclampsia is a multifactorial and pathogenic pathway syndrome. The present study compared the effects of Pra on clinical manifestations of preeclampsia in different pathogenic pathways.

Methods: Two different preeclampsia-like mouse models used in this study were generated with No-nitro-L-arginine methyl ester (L-NAME) and used lipopolysaccharide (LPS) from day 7 of gestation, respectively. Pra treatment was administered on day 2 after the models were established in each group (L-NAME + Pra, LPS + Pra, and Control + Pra, $n = 8$) or normal saline (NS) for the control group (L-NAME + NS, LPS + NS, and Control + NS, $n = 8$). Maternal weight, serum lipids, the histopathological changes, and lipid deposition in the liver and placenta were observed. The pregnancy outcomes were compared. The blood pressure analysis was carried out on repeated measurements of variance. Student's *t*-test was used for comparing the two groups. The enumeration data were compared by Chi-square test.

Results: The mean arterial pressure (MAP) and 24-h urinary protein in the L-NAME + NS and LPS + NS groups were significantly higher than the Control + NS group ($F = 211.05$ and 309.92 for MAP, $t = 6.63$ and 8.63 for 24-h urinary protein; all $P < 0.05$) and reduced in the L-NAME + Pra group as compared to the L-NAME + NS group ($F = 208.60$ for MAP, $t = 6.77$ for urinary protein; both $P < 0.05$). Urinary protein was decreased in the LPS + Pra group as compared to the LPS + NS group ($t = 5.33$; $P < 0.05$), whereas MAP had no statistical significance ($F = 3.37$; $P > 0.05$). Compared to the Control + NS group, the placental efficiency in the L-NAME + NS and LPS + NS groups decreased significantly ($t = 3.09$ and 2.89 , respectively; both $P < 0.05$); however, no significant difference was observed in L-NAME + Pra and LPS + Pra groups ($t = 1.37$ and 0.58 , respectively; both $P > 0.05$). Free fatty acid was elevated in the L-NAME + NS group as compared to the Control + NS group ($t = 3.99$; $P < 0.05$) at day 18 of pregnancy and decreased in the L-NAME + Pra group as compared to the L-NAME + NS group ($t = 3.28$; $P < 0.05$); however, no significant change was observed in the LPS model ($F = 0.32$; $P > 0.05$).

Conclusion: This study suggested that Pra affected the clinical manifestations differently in preeclampsia-like mouse models generated in various pathogenic pathways.

Key words: Lipids; Mouse; Pravastatin; Preeclampsia

INTRODUCTION

Preeclampsia is a pregnancy complication syndrome that affects 3–5% of pregnancies and is characterized by new-onset hypertension and proteinuria after 20 weeks of gestation or dysfunction of maternal organs and systems. However, only limited prevention or treatment of preeclampsia is plausible as the precise pathogenesis of the disease is yet unclear. The current preventive measures, such as calcium, cod liver oil, antioxidants, low-dose aspirin (LDA), heparin, and diet or lifestyle interventions, show potential but small benefits. Pravastatin (Pra) belongs to the statins that possess lipid-lowering properties and efficiency in reducing morbidity and

mortality of cardiovascular disease by inhibiting the 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA) reductase. In addition, Pra exhibits several cholesterol-independent effects such as vasodilator and anti-inflammatory effects, inhibition of anti-angiogenic factors, and upregulation of endothelial nitric oxide

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synthases, thereby rendering them physiologically plausible for the prevention of preeclampsia.

The recent studies have shown that Pra can lower the blood pressure and improve proteinuria in some preeclampsia-like rodent models including the reduced uteroplacental perfusion pressure (RUPP)-induced rat model and the anti-angiogenic factor-soluble fms-like tyrosine kinase (sFlt-1)-induced mouse model or the complement component 1Q deletion.^[1-3] The results of a clinical pilot study conducted by Brownfoot *et al.*^[4] included four cases; the results showed that the application of Pra might affect the alleviation of the development of preeclampsia. Lefkou *et al.*^[5] found that the addition of Pra, based on the LDA and low-molecular-weight heparin in pregnant women, who are suffering from antiphospholipid syndrome and are likely to develop preeclampsia or intrauterine growth restriction, can improve the outcome of pregnancy. Furthermore, other cases reported that the application of Pra could restore the balance of angiogenesis and prevent recurrent fetal death in massive perivillous fibrin deposition of the placenta.^[6] A previous study on the mechanism of Pra in animal models addressed the regulation of the angiogenic factors, improvement of vascular endothelial cell function, reduction of oxidative stress, and the regulation of the immune system.^[7]

Interestingly, Pra exerts protective effects in preeclampsia both in some rodent models and several clinical cases;^[1-4] however, multiple factors of pathogenesis and pathogenic pathways exist in preeclampsia.^[8] Thus, whether Pra has a positive effect on all the clinical preeclampsia is yet to be elucidated. Herein, we observed and compared the effect of Pra on the clinical manifestations in two preeclampsia-like mouse models induced by different approaches. N ω -nitro-L-arginine methyl ester (L-NAME) is an endothelial nitric oxide synthase inhibitor, and our previous study showed that injecting L-NAME into C56BL/6J mice could induce preeclampsia-like symptoms such as hypertension and proteinuria, as well as long-chain fatty acid oxidation (FAO) disorders. On the other hand, abnormal lipid metabolism was not detected in the ultra-low-dose lipopolysaccharide (LPS)-induced preeclampsia-like mouse model, which might be mediated directly by the inflammatory pathway.^[9] These results suggested that different pathogens exist in the preeclampsia models established in different pathways. In the present study, we established different preeclampsia-like models and detected their clinical manifestations and lipid metabolism after administration of Pra in order to further evaluate whether the drug is suitable for the prevention or treatment in the multifactor-related preeclampsia.

METHODS

Experimental animals

The animal experiments were approved by the Animal Ethics Committee of Peking University Health Science

Center. Wild-type C57BL/6J mice were purchased from the Laboratory Animal Science Department of Peking University Health Science Center. Female mice aged 8–10 weeks and male mice aged 10–14 weeks were reared in a barrier environment at a temperature of $23 \pm 2^\circ\text{C}$, relative humidity of $55 \pm 10\%$, 12 h light-dark cycle, and free drinking water. The mice were mated at a ratio of 2:1 (female:male). Females were inspected daily for vaginal plugs and designated as gestational on day 1.

Establishment and intervention of mouse models

The preeclampsia-like model was established through the following methods: (1) L-NAME model: L-NAME (Sigma-Aldrich, St. Louis, MO, USA) $50 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ was administered subcutaneously to pregnant mice from the gestational day 7 to 18 and (2) LPS model: the pregnant mice were injected a single intraperitoneal injection with an ultra-low dose of LPS (Sigma-Aldrich, St. Louis, MO, USA), $1 \mu\text{g}/\text{kg}$ on the gestational day 7. Pregnant mice were simultaneously injected with saline as a normal pregnancy control group (Control). The pregnant mice of each group were randomly divided into two groups treated with normal saline (NS) (L-NAME + NS, LPS + NS, and Control + NS, $n = 8$) or Pra (Sigma-Aldrich) $5 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ (L-NAME + Pra, LPS + Pra, and Control + Pra, $n = 8$) from days 8–18 of gestation by intragastric administration. L-NAME or LPS was injected into nonpregnant mice as the nonpregnant control group (NP-L-NAME and NP-LPS, $n = 8$).

Mean arterial pressure and 24-h urinary protein

Blood pressure was measured every 2 days with a CODA noninvasive tail-cuff system (Kent Scientific Corporation, Washington, DC, USA) until the last day of gestation. The mice were placed in a fixator with the tail exposed, and the CODA system measured the blood flow in the tail during each measurement cycle and deduced the mean arterial pressure (MAP). Each measurement comprised of five adaption cycles and ten measuring cycles; the average MAP level of the measurement cycles is used for the statistical analysis of the data. Each mouse was placed in a standard metabolic cage on day 17, and urine samples were collected every 24 h. The urine protein was determined by the protein assay kit I (Bio-Rad, Hercules, CA, USA). The experimental procedures performed were according to the product specifications.

Sample collection

The blood samples were withdrawn on the gestational days 8 and 18 and centrifuged at 2000 g for 10 min to obtain serum samples that are stored at -80°C . The pregnant mice were sacrificed after anesthesia by intraperitoneal injection of 10% chloral hydrate at a dose of $3 \text{ ml}/\text{kg}$ (Sigma-Aldrich) on day 18 of pregnancy. A cesarean section was performed, and the number of live births and absorbed fetus, placental, and fetal wet weights were recorded. A part of the maternal liver and placenta was fixed with 4% neutral formaldehyde partially in optimal cutting temperature (OCT) compound for frozen oil red O staining.

Blood lipid levels

Serum free fatty acid (FFA) levels were measured using the NEFA test kit (Wako Chemicals, Japan). The levels of triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were measured by an automatic biochemical analyzer using a blood lipid detection kit (Sekisui, Japan), according to the manufacturer's recommendations.

Histopathological and lipid deposition detection

Maternal liver and placenta tissues fixed in 4% formaldehyde were dehydrated and embedded in paraffin and cut into 5 μm slices, followed by hematoxylin and eosin (H and E staining) staining; the morphological changes were observed and images were acquired using NIS-Elements (Nikon, Japan). The liver and placental tissues of the embedded OCT were sliced into 10 μm with a frozen slicer and stained with an oil red O staining kit (GenMed Scientific, Wilmington, DE, USA). The images were analyzed using Image-Pro Plus 6.0 (Media Cybernetics, MD, USA). Five randomly selected fields (original magnification, $\times 400$) were detected in each sample, the results of red stained area/total area were collected, and the average of five results was analyzed for each sample ($n = 8$ samples/group).

Statistical analysis

The data of MAP, proteinuria, maternal weight on the gestational day 18, fetal and placental weight, placental efficiency (fetal weight/placental weight), number of live and absorbed births, serum FFA, TG, TC, LDL, HDL levels, and the lipid deposition area were analyzed with SPSS version 20.0 (SPSS Inc., Chicago, IL, USA), and the figures were illustrated by Prism software (GraphPad Software Inc., CA, USA). The data were expressed as the mean \pm standard deviation (SD). The MAP of each group was analyzed by repeated measurement analysis of variance (ANOVA); the independent-sample t -test and one-way ANOVA were performed on the quantitative data. Enumeration data were analyzed by Chi-square test. Differences were statistically significant when $P < 0.05$.

RESULTS

Identification of two preeclampsia-like models

L-NAME + NS and LPS + NS model groups showed preeclampsia-like changes of hypertension and proteinuria after the gestational day 7, indicating that the two preeclampsia-like models were established successfully.

The MAP was significantly higher in the L-NAME + NS and LPS + NS groups than that in the Control + NS group from the gestational days 8–18 ($F = 211.05$ and 309.92 , respectively; both $P < 0.05$). However, no significant differences were observed in the MAP between the L-NAME + NS and LPS + NS groups ($F = 3.23$; $P > 0.05$). Compared to the corresponding nonpregnant NP-L-NAME and NP-LPS groups, the MAP in L-NAME + NS and LPS + NS groups was significantly higher from the gestational day 8 ($F = 274.94$ and 1032.73 , respectively; both $P < 0.05$).

No significant differences were noted in the MAP between the NP-L-NAME and NP-LPS groups as compared to the Control + NS group [$F = 0.80$ and 0.06 , respectively; both $P > 0.05$, Figure 1a].

Compared to the Control + NS group, the levels of 24-h urinary protein increased significantly in the L-NAME + NS and LPS + NS groups ($t = 6.63$ and 8.63 , respectively; both $P < 0.05$); no significant differences were observed between the L-NAME + NS and LPS + NS groups ($t = 0.97$; $P > 0.05$). In addition, these levels increased significantly in the L-NAME + NS and LPS + NS groups as compared to the corresponding nonpregnant control group ($t = 6.58$ and 9.52 , respectively; both $P < 0.05$). Furthermore, no significant differences were evident in the NP-L-NAME and NP-LPS groups in the 24-h urinary protein levels as compared to the Control + NS group [$t = 1.09$ and 0.99 , respectively; both $P > 0.05$, Figure 1c].

Effect of pravastatin on mean arterial pressure and proteinuria in the two models

Compared to the L-NAME + NS group, the MAP in the L-NAME + Pra group decreased significantly ($F = 208.60$; $P < 0.05$); however, the levels in the L-NAME + Pra group were higher than those that in the Control + NS group ($F = 35.41$; $P < 0.05$). No significant difference was observed in the MAP between the LPS + Pra and LPS + NS groups ($F = 3.37$; $P > 0.05$). The MAP in the L-NAME + Pra group decreased significantly as compared to the LPS + Pra group [$F = 109.46$; $P < 0.05$, Figure 1b].

The level of proteinuria decreased significantly in the L-NAME + Pra group as compared to L-NAME + NS ($t = 6.77$; $P < 0.05$), and there was no significant difference between the L-NAME + Pra and the Control + NS groups ($t = 0.62$; $P > 0.05$). Compared to LPS + NS, the level of proteinuria decreased significantly in the LPS + Pra group ($t = 5.33$; $P < 0.05$); however, the level elevated significantly in the LPS + Pra group as compared to the Control + NS group ($t = 3.20$; $P < 0.05$). The level of proteinuria in the LPS + Pra group was significantly higher than that in the L-NAME + Pra group [$t = 3.08$; $P < 0.05$, Figure 1d].

Pregnancy outcome

The fetal and placental wet weight: compared to the Control + NS group, the average weight of the fetus and placenta in the L-NAME + NS and LPS + NS groups decreased significantly ($t = 5.41$ and 5.31 for fetus, $t = 3.97$ and 3.56 for placenta, respectively; all $P < 0.05$), and no significant difference was observed between the L-NAME + NS and LPS + NS groups ($t = 0.87$ for fetus, $t = 0.72$ for placenta; all $P > 0.05$). Compared to the corresponding L-NAME + NS and LPS + NS groups, no significant difference was noted in the L-NAME + Pra and LPS + Pra groups after the administration of Pra ($t = 1.40$ and 0.34 for fetus and $t = 1.00$ and 1.05 for placenta, respectively; all $P > 0.05$), while the L-NAME + Pra and LPS + Pra groups did not vary significantly ($t = 0.49$ for fetus and 0.76 for placenta; both $P > 0.05$). Furthermore, no significant difference occurred between the Control + Pra and

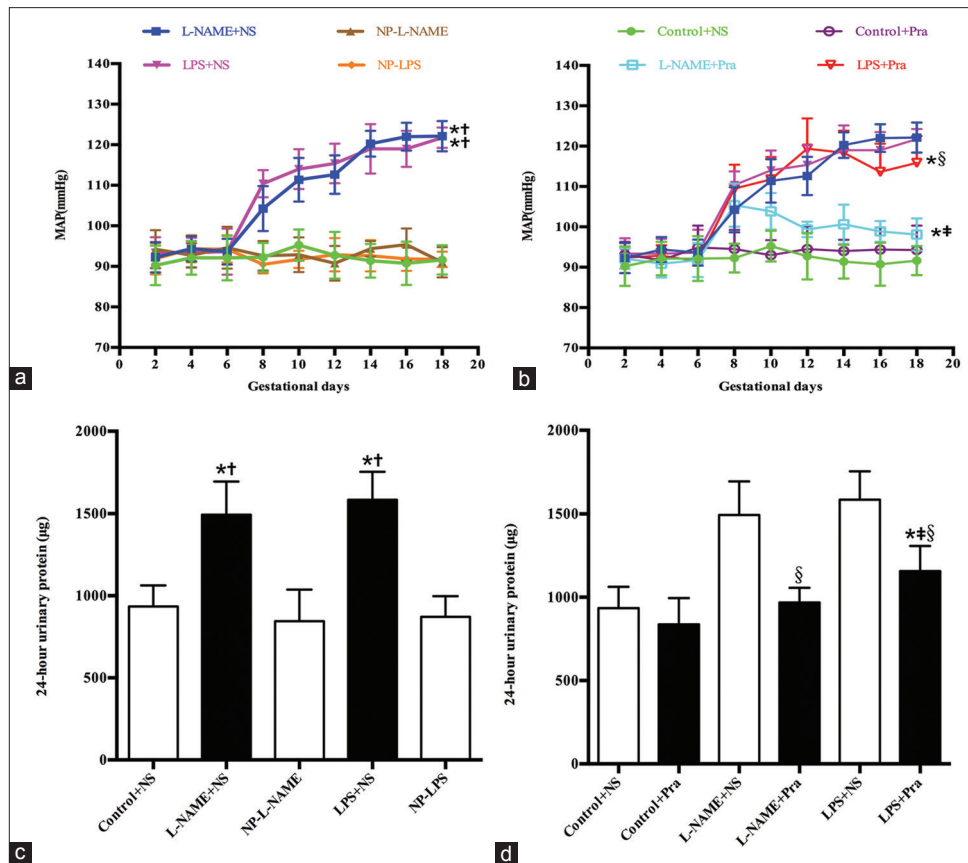


Figure 1: The MAP (a) and 24-h urinary protein levels (c) in the two models induced by L-NAME or LPS injection and control groups, and the changes of MAP (b) and urinary protein levels (d) after application of pravastatin in two models. * $P < 0.05$, compared with Control + NS group, [†] $P < 0.05$, compared with corresponding NP group. [‡] $P < 0.05$, compared with L-NAME + Pra group, [§] $P < 0.05$, compared with corresponding + NS group. Data were expressed as mean \pm SD and $n = 8$ per group. 1 mmHg = 0.133 kPa. MAP: Mean arterial pressure; L-NAME: N ω -nitro-L-arginine methyl ester; LPS: Lipopolysaccharide; NS: Normal saline; Pra: Pravastatin; NP: Nonpregnant; SD: Standard deviation.

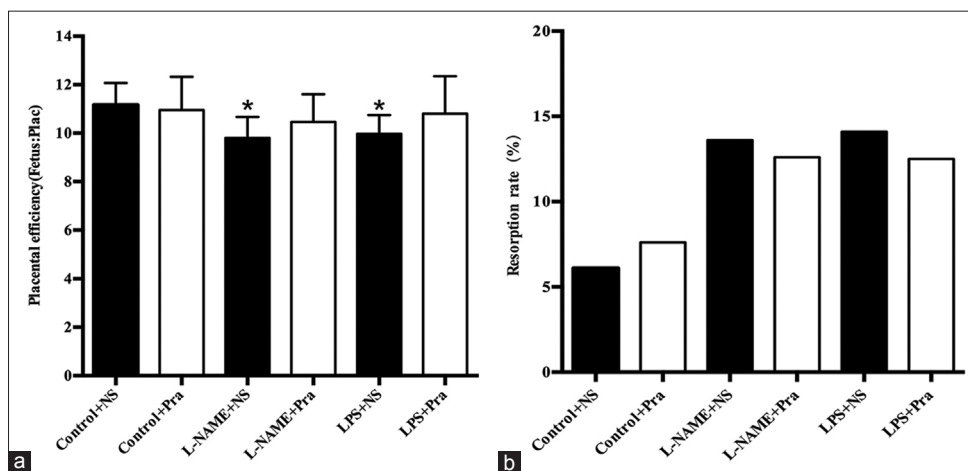


Figure 2: Effects of pravastatin on placental efficiency and resorption rate. Placental efficiency in all groups (a) and resorption rate in all groups (b). * $P < 0.05$, compared with Control + NS. Placental efficiency data were expressed as mean \pm SD, the resorption rate was obtained by (the total number of absorbed fetuses per group)/(the total number of births per group), $n = 8$ per group. LPS: Lipopolysaccharide; NS: Normal saline; Pra: Pravastatin; L-NAME: N ω -nitro-L-arginine methyl ester; SD: Standard deviation.

Control + NS groups ($t = 1.22$ for fetus and 0.61 for placenta; both $P > 0.05$). The changes in the maternal weight on the gestational day 18 were same as the fetal and placental wet weight [Table 1].

Placental efficiency

Compared to the Control + NS group, the placental efficiency of the L-NAME + NS and LPS + NS groups significantly decreased ($t = 3.09$ and 2.85, respectively; both

Table 1: The maternal, fetal, and placental weight and the number (rate) of live and absorbed fetuses in all groups (n = 8 per group)

Groups	Maternal weight (g)	Fetal weight (g)	Placental weight (mg)	Live fetuses	Absorbed fetuses
Control + NS	31.84 ± 1.01	1.03 ± 0.11	92.5 ± 8.86	62 (93.9)	4 (6.1)
Control + Pra	31.46 ± 2.50	0.98 ± 0.05	90.0 ± 7.56	61 (92.4)	5 (7.6)
L-NAME + NS	29.51 ± 1.61*	0.75 ± 0.10*	76.3 ± 7.44*	57 (86.4)	9 (13.6)
L-NAME + Pra	29.80 ± 0.95*	0.81 ± 0.07*	77.5 ± 7.07*	55 (87.3)	8 (12.6)
LPS + NS	29.45 ± 0.95*	0.78 ± 0.07*	78.8 ± 6.41*	55 (85.7)	9 (14.1)
LPS + Pra	30.11 ± 0.77*	0.83 ± 0.11*	77.5 ± 10.35*	56 (87.5)	8 (12.5)
F	4.17	6.91	13.66		0.91
P	<0.05	<0.05	<0.05		0.48

The data of maternal, fetal, and placental weight were shown as mean ± SD, the number and rate of live and absorbed fetus were shown as n (%). * $P < 0.05$, compared with control + NS. L-NAME: N ω -nitro-L-arginine methyl ester; LPS: Lipopolysaccharide; NS: Normal saline; Pra: Pravastatin; SD: Standard deviation.

$P < 0.05$), and no significant difference was noted in the L-NAME + NS and LPS + NS groups ($t = 0.42$; $P > 0.05$). Although the L-NAME + Pra and LPS + Pra groups did not show any statistically significant difference as compared to the corresponding L-NAME + NS and LPS + NS groups ($t = 1.30$ and 1.35 , respectively; both $P > 0.05$), no significant difference was observed in the L-NAME + Pra and LPS + Pra groups as compared to the Control + NS group ($t = 1.37$ and 0.58 ; both $P > 0.05$). Furthermore, no significant difference was observed in the L-NAME + Pra and LPS + Pra groups ($t = 0.49$; $P > 0.05$) and similar result was noted between the Control + Pra and the Control + NS groups [$t = 0.37$; $P > 0.05$, Figure 2a].

Number of live and absorbed fetuses

No statistically significant difference was noted between the number of live births and absorbed fetuses ($\chi^2 = 3.83$, $P > 0.05$). The resorption rate increased in the L-NAME + NS and LPS + NS groups as compared to the Control + N group; however, the difference was not significant ($t = 1.59$ and 1.45 , respectively; both $P > 0.05$). After the application of Pra, the resorption rate in the L-NAME + Pra and LPS + Pra groups was slightly lower than the L-NAME + NS and LPS + NS groups, although the difference was not significant [$t = 0.36$ and 0.26 , respectively; both $P > 0.05$, Figure 2b].

Blood lipid concentration

FFA levels: No significant difference was observed in serum FFA levels in all groups on the gestational day 8, and none was observed between the gestational days 8 and 18 in each group except the L-NAME + NS group ($F = 0.61$; $P > 0.05$). The FFA levels during pregnancy on day 18 increased significantly in the L-NAME + NS group as compared to the Control + NS group ($t = 3.99$; $P < 0.05$) and significantly decreased in the L-NAME + Pra group as compared to the L-NAME + NS group ($t = 3.28$; $P < 0.05$). There was no difference in the L-NAME + Pra group as compared to the Control + NS group ($t = 0.69$; $P > 0.05$), and no significant differences were observed in the levels of FFA in the LPS model ($F = 0.32$; $P > 0.05$). The FFA levels were elevated in the L-NAME + NS group on day 18 as compared to the LPS + NS group ($t = 4.96$; $P < 0.05$), and no significant

difference was observed in the L-NAME + Pra group as compared to the LPS + Pra group ($t = 1.30$; $P > 0.05$). Furthermore, no significant difference was noted in the FFA levels between the Control + Pra and Control + NS groups [$t = 0.29$; $P > 0.05$, Figure 3a].

There were no significant differences in the CHO, TG, LDL, and HDL levels in all groups on both the gestational days, 8 and 18 ($F = 0.51$ for CHO, 0.23 for TG, 1.01 for LDL, and 1.57 for HDL; all $P > 0.05$, [Figure 3b–3e]).

Liver and placental morphological changes

H and E staining showed regular liver morphology in the Control + NS group with ordered hepatocytes and evenly distributed cytoplasm. In addition, vacuolization was noted between the hepatocytes in the L-NAME + NS group suggesting hepatic steatosis, which disappeared in the L-NAME + Pra group. No obvious changes in the steatosis in the LPS model were noted [Figure 4a].

The results of placental H and E staining showed that as compared to the Control + NS group, the L-NAME + NS group exhibited reduced maternal placental sinus and loose placental tissue in the spongiotrophoblast and labyrinth zone; these parameters were alleviated in the L-NAME + Pra group and did not exist in the LPS model [Figure 4b].

Lipid deposition in liver and placenta

Oil red O staining showed that the area of lipid deposition in the liver and placenta significantly increased in L-NAME + NS groups as compared to the Control + NS group ($t = 9.12$ for liver, 16.7 for placenta, both $P < 0.05$), and the lipid deposition of placenta was mainly distributed in the spongiotrophoblast of the pregnant mice. Compared to the L-NAME + NS group, the deposition area of the L-NAME + Pra group was significantly lower ($t = 6.55$ for liver, 10.53 for placenta, both $P < 0.05$); however, the area in the L-NAME + Pra group was still significantly higher than that of the Control + NS group ($t = 4.80$ for liver, 6.33 for placenta, both $P < 0.05$). The lipid deposition area in the liver was significantly higher in the LPS + NS group as compared to the Control + NS group ($t = 3.37$, $P < 0.05$) and did not exist in the placenta ($t = 1.82$, $P > 0.05$). Compared to the

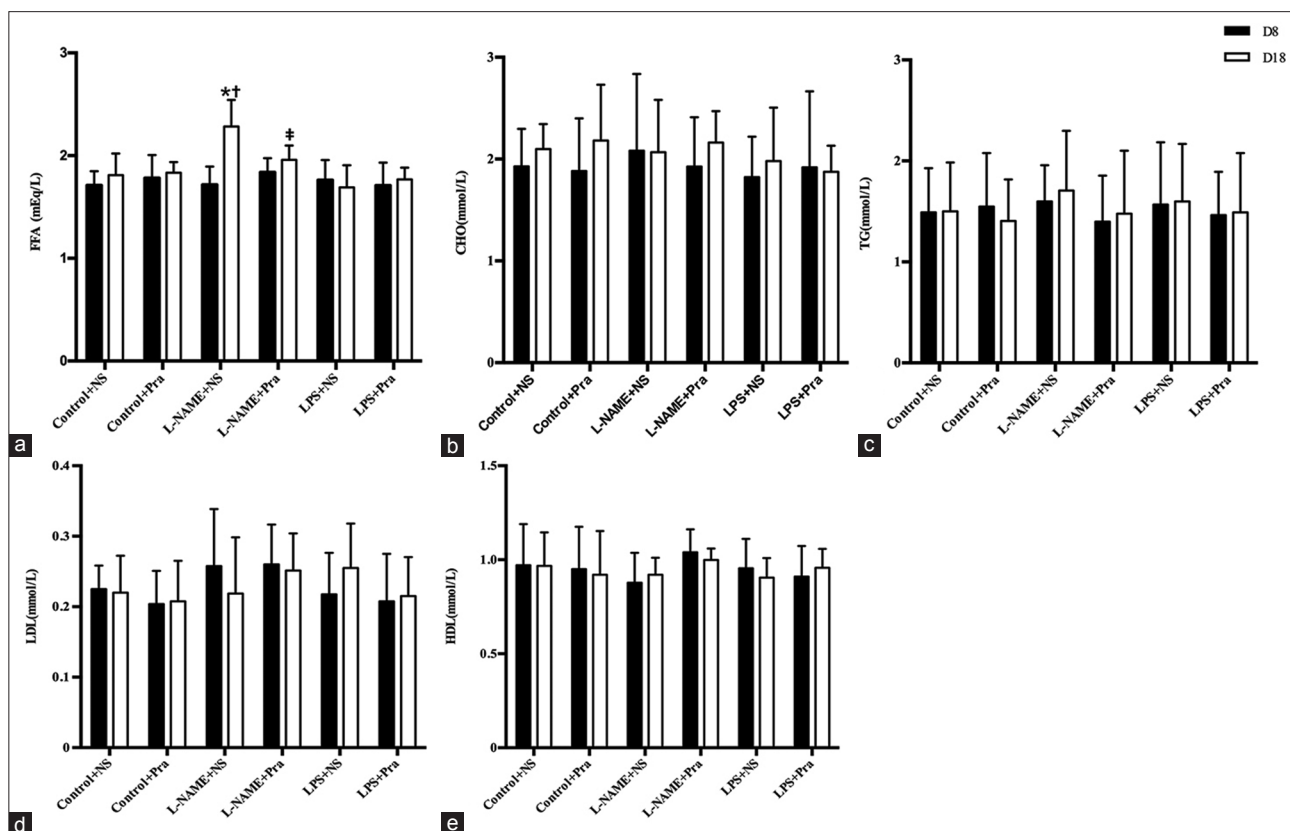


Figure 3: Serum lipid concentrations in all groups. FFA (a), CHO (b), TG (c), LDL (d), and HDL (e) concentrations. * $P < 0.05$, compared with corresponding gestational D8th serum levels, † $P < 0.05$, compared with Control + NS of D18. ‡ $P < 0.05$, compared with L-NAME + NS of D18. Data were expressed as mean \pm SD and $n = 8$ per group. FFA: Free fatty acid; CHO: Cholesterol; TG: Triglyceride; LDL: Low-density lipoprotein; HDL: High-density lipoprotein concentrations; LPS: Lipopolysaccharide; NS: Normal saline; Pra: Pravastatin; L-NAME: *N* ω -nitro-L-arginine methyl ester; SD: Standard deviation.

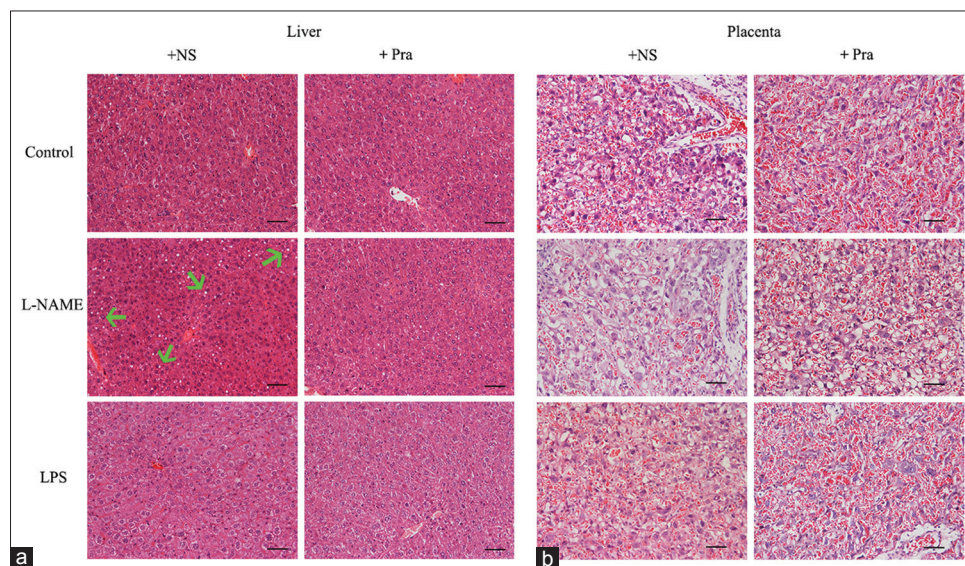


Figure 4: Morphological changes in the maternal liver (a) and placenta (b) at day 18 of pregnancy in all groups (hematoxylin and eosin staining, scale bars 50 μ m); liver and placenta arrows refer to the vacuolization between the hepatocytes. LPS: Lipopolysaccharide; NS: Normal saline; Pra: Pravastatin; L-NAME: *N* ω -nitro-L-arginine methyl ester; SD: Standard deviation.

LPS + NS group, the LPS + Pra group showed a decreased lipid deposition area in the liver but the difference was not statistically significant ($t = 1.70$, $P > 0.05$). The areas in both the liver and placenta in the L-NAME + NS group

were larger than that the LPS + NS group ($t = 7.14$ for liver, 15.87 for placenta, both $P < 0.05$), and the L-NAME + Pra lipid deposition area was significantly higher than that of the LPS + Pra group ($t = 3.94$ for liver, 5.13 for placenta,

$P < 0.05$). Moreover, no significant difference was observed in the Control + Pra group as compared to the Control + NS group ($t = 0.29$ for liver, 0.73 for placenta, $P > 0.05$, [Figure 5]).

DISCUSSION

Pra is a competitive inhibitor of HMG-CoA reductase, a key enzyme in cholesterol synthesis that regulates CHO, LDL, VLDL, and TG serum levels through direct or indirect pathways. The efficiency of Pra can reduce the morbidity and mortality from cardiovascular disease, which has been estimated previously,^[10] some of which have shown that it can exert anti-inflammatory, free radical reduction, upregulation of endothelial nitric oxide synthase, inhibition of smooth muscle cell proliferation, and immune regulation.^[11] Preeclampsia and cardiovascular disease share similar risk factors and pathogenesis. Endothelial dysfunction is the basic pathogenesis of preeclampsia and atherosclerosis, and the multiefficiency of Pra displayed in the cardiovascular system rendered it as the potential drug for the prevention or treatment of preeclampsia. Although Pra can improve the clinical manifestations of preeclampsia in animal models

and small sample studies, Odiari *et al.*^[12] found that Pra did not alleviate the placental trophoblastic damage induced by anti-phospholipid antibodies (aPL); on the contrary, it amplified the aPL-induced inflammatory reaction. Thus, the effect of Pra in aPL-related preeclampsia prevention may be relatively limited, which further suggested the different effects on preeclampsia patients, who might exhibit multiple factors and pathogenic pathways.

In order to observe and compare the effects of Pra in different models, we established two preeclampsia-like mouse models induced by two different factors: the L-NAME model based on the inhibition of the synthesis of vascular endothelial factor nitric oxide and the LPS model based on triggering the inflammatory action caused by an ultra-low-dose LPS. The application of Pra in both models revealed that some preeclampsia-like symptoms were alleviated, including the decrease of MAP and hepatic and placental damage in the L-NAME model, as well as the improvement of proteinuria and pregnancy outcomes in both models.

Although the current research results confirmed that Pra can alleviate some of the preeclampsia-like symptoms, it had different effects on the clinical manifestations of

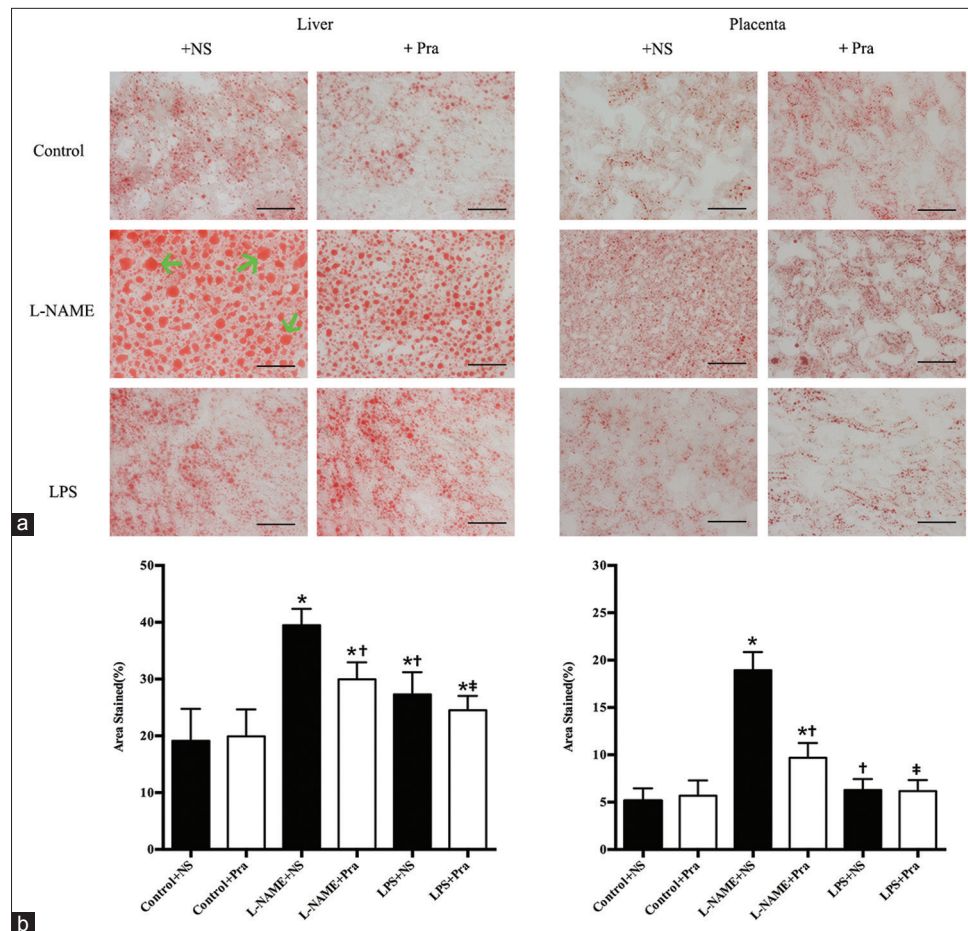


Figure 5: Lipid depositions in hepatic and placental tissues with oil-red O staining (a) (scale bars 50 μ m) and the analyzed percentage of lipid droplets stained area (b). * $P < 0.05$ compared with Control + NS, † $P < 0.05$ compared with L-NAME + NS, ‡ $P < 0.05$, compared with L-NAME + Pra group. Data are shown as mean \pm SD, $n = 8$ per group; arrows refer to the increased lipid droplets. LPS: Lipopolysaccharide; NS: Normal saline; Pra: Pravastatin; L-NAME: $N\omega$ -nitro-L-arginine methyl ester; SD: Standard deviation.

preeclampsia in the two models. Pra can reduce the MAP and urine protein levels and improve the hepatic and placental pathological damage in the L-NAME model. In the LPS model, we observed that only the urinary protein levels were reduced, whereas MAP and the pathological changes in the liver and placenta were not alleviated distinctly. This imbalance might be related to the different pathogenic factors in the two models. The previous study has demonstrated different pathogenic pathways in the L-NAME- and LPS-induced preeclampsia-like models. The pathogenesis in the L-NAME model may related to FAO disorders, whereas the LPS model might present another pathogenic way.^[9] In the L-NAME model, the FAO disorders occur, with decreased levels of mRNA and protein expression of long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), and FFA levels were negatively correlated with LCHAD levels. On the other hand, this phenomenon might be directly mediated by the inflammatory pathway in the LPS model induced by inflammatory cytokines through the activation of the NF- κ B signaling pathway, leading to activation and damage of endothelial cells.^[13] The experimental results demonstrated elevated levels of FFA in the L-NAME model, while the phenomenon was not observed in the LPS model. Moreover, the lipid deposition in the liver and placenta in the L-NAME model was heavier than that in the LPS model, which was in agreement with our previous study results.

Nevertheless, the mechanism of Pra is yet unclear. Kumasawa *et al.*^[2] observed that after the application of Pra in the sFlt-1-induced preeclampsia-like mouse model, the symptoms of hypertension and proteinuria were eased, fetal growth restriction improved, sFlt-1 levels decreased, placental growth factor (PLGF) levels increased, and the endothelial cell proliferation improved. These parameters substantiated the protective effects of Pra on preeclampsia, which is also related to the induction of PLGF by a placenta-specific PLGF expression. In L-NAME-induced preeclampsia-like rat models, sildenafil citrate can reduce the gestational hypertension and proteinuria and improve the pregnancy outcomes by reducing the plasma levels of sFlt-1 and sEng.^[14] Another study showed that the application of Pra in the RUPP preeclampsia-like model^[1] reduced the increased blood pressure and urinary protein, improved the outcome of pregnancy, and regulated the balance of sFlt-1/vascular endothelial growth factor. These results suggested that the role of Pra in preeclampsia-like models might be related to its regulation of angiogenic factors. In addition, the protective effects of Pra in preeclampsia include regulating the oxidative stress,^[1] relieving the oxidative stress by promoting the expression of HO-1, regulating the vascular endothelial function by elevating nitric oxide synthase,^[15] reducing the inflammation, and relieving the placental and fetal damage by inhibiting the activation of complement cascade.^[16] In this study, we speculated that the mechanisms of Pra involved in the L-NAME model might be mediated through FAO regulation.

The current study demonstrated that abnormal lipid metabolism was associated with the onset of preeclampsia^[17]

with an increase in TG, CHO, and FFA levels; these phenomena might be associated with several pathogenesis in preeclampsia patients.^[18,19] The *in vitro* experiments revealed that elevated FFA levels were associated with the decreased invasive ability of trophoblastic cells^[20] and that increased FFA can induce the release of reactive oxygen species and activate nitrogen (RNS), which in turn, leads to the activation of an inflammatory signaling pathway causing inflammatory responses.^[21] A previous study had also shown that the elevated levels of FFA in plasma can lead to insulin resistance, which is correlated with preeclampsia.^[22] Thus, long-chain FAO disorders leading to the elevated levels of FFA are involved in some preeclampsia condition. In this study, Pra exerted different effects on lipid metabolism in the two models. In the L-NAME model, Pra reduced the plasma FFA levels in pregnant mice and reduced the lipid deposition in the liver and placenta, whereas Pra did not demonstrate any of these effects in the LPS model. Currently, the regulation of FFA by statins is controversial. A study has shown that atorvastatin can reduce liver and plasma FFA levels in rats,^[23] and a meta-analysis of clinical, randomized controlled trials showed that statins can reduce the plasma FFA levels in patients with hyperlipidemia, thereby improving the prognosis of patients with cardiovascular disease.^[24] An animal study has shown that statins can prevent mice from nonalcoholic fatty liver and promote FAO in liver mitochondria and peroxisomes.^[25] However, a small sample study found that Pra^[26] or high-dose simvastatin^[27] in type 2 diabetes patients did not decrease the FFA levels significantly. The study on statins displayed different effects on FFA levels, indicating that the mechanism underlying their activities might be affected by a variety of factors. Herein, the different effects of Pra on FFA levels may be related to the different pathogenesis in the two models, and whether the different effects of Pra on the clinical manifestations in the two models are associated with the effects on lipid levels necessitates further investigation.

Pra, as a classical lipid-lowering drug, had no significant effect on TG, TC, HDL, and LDL serum levels in pregnant mice; however, it improved the preeclampsia-like symptoms. Fox *et al.*^[15] found that Pra did not alter the cholesterol levels in the sFlt-1-induced model; however, it can promote a nitric oxide synthase expression and improve vascular function, suggesting that the protective effects of Pra in preeclampsia-like pregnant mice may act as a cholesterol-independent pathway. Thus, we speculated that the lipid-lowering effect of Pra in our model is not obvious, and that it may be related to the baseline of the lipid levels of mice and the duration or dose of the drug. The effects of statins may be mediated by the inhibition of small molecule G protein activation. The intermediate products including mevalonate, farnesyl pyrophosphate, and geranylgeranyl pyrophosphate in cholesterol synthesis are critical for the posttranslational modification of small G proteins such as the Rho, Ras, Rac, and the nuclear lamina layer protein. The activated G protein transfers from the cell cytoplasm to membrane and regulates the cell functions. Thus, statins

may play a variety of roles in several signaling pathways via their effect on small G proteins.

The National Institute for Child Health and Human Development conducted a study applying Pra in pregnant women with a high risk of preeclampsia after approval from the US Food and Drug Administration (FDA); the study provided preliminary data on the safety and pharmacokinetics of Pra application.^[28] The current study showed that Pra was relatively safe in pregnancy even though FDA prescribed statins for pregnancy category X, and current recommendations suggested discontinuation of the medication immediately in the event of pregnancy or before conception.^[29,30] However, the use of other statins (cerivastatin, simvastatin, lovastatin, or atorvastatin) might be associated with an increase in the incidence of skeletal malformations which was attributed to the difference in the physicochemical properties between the hydrophilic Pra and the other lipophilic statins.^[31] Recent animal experiments showed that Pra exerted protective effects in maternal preeclampsia and fetal mice.^[32,33] *In vitro* experiments displayed that Pra did not have any effect on the physiological function in a normal human placenta;^[34] also, there was no clinical evidence of Pra toxicity on the embryo. The ongoing Phase II randomized clinical trials of statins to ameliorate early-onset preeclampsia (StAmP) for the use of Pra in preeclampsia will also provide us with further clinical data on the drug.

In conclusion, the study showed that Pra exhibited different effects on preeclampsia-like clinical manifestations and lipid metabolism in two preeclampsia models, thereby suggesting that the drug may have different effects and mechanisms in multifactorial and multipathogenic preeclampsia. Whether Pra is suitable for the prevention and treatment of preeclampsia with the characteristics of multiple factors, pathogenesis, and pathogenic pathways necessitates further investigation.

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Conflicts of interest

There are no conflicts of interest.

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普伐他汀对不同小鼠模型中子痫前期样表现的不同影响

摘要

目的: 普伐他汀 (pravastatin, Pra) 在子痫前期中具有保护作用, 但子痫前期是一种多因素, 多致病通路的综合征, 本研究旨在观察及比较普伐他汀对不同致病因素的子痫前期临床表现的影响。

方法: 自孕7天开始分别注射左硝基精氨酸甲酯 (N ω -nitro-L-arginine methyl ester, L-NAME) 及脂多糖 (lipopolysaccharide, LPS) 建立两种子痫前期样小鼠模型, 建模第2天用普伐他汀 (L-NAME + Pra, LPS + Pra, Control + Pra, $n = 8$) 进行灌胃, 生理盐水 (L-NAME + NS, LPS + NS, Control + NS, $n = 8$) 作为对照。观察及比较各组小鼠妊娠结局, 血脂水平, 肝脏和胎盘病理形态学改变及脂质沉积情况。血压值用重复测量方差分析方法进行分析, 计量资料两组间比较用 t 检验, 计数资料用卡方检验进行分析。

结果: 平均动脉压及24小时尿蛋白水平在L-NAME + NS, LPS + NS组比Control + NS组显著升高 (血压值 $F = 211.05, 309.92$, 尿蛋白水平 $t = 6.63, 8.63$; $P < 0.05$), 在L-NAME + Pra组与L-NAME + NS组相比降低 (血压值 $F = 208.60$, 蛋白尿 $t = 6.77$; $P < 0.05$)。LPS + Pra组与LPS + NS组相比, 蛋白尿水平降低 ($t = 5.33$; $P < 0.05$), 而平均动脉压无显著差异 ($F = 3.37$, $P > 0.05$)。与Control + NS组相比, 胎盘效率在L-NAME + NS组, LPS + NS组降低 ($t = 3.09, 2.89$; $P < 0.05$); 而在L-NAME + Pra组, LPS + Pra组无显著性差异 ($t = 1.37, 0.58$; $P > 0.05$)。孕18天游离脂肪酸水平在L-NAME + NS组显著高于Control + NS组 ($t = 3.99$; $P < 0.05$), 而在L-NAME + Pra组比L-NAME + NS组显著降低 ($t = 3.28$; $P < 0.05$), 在LPS模型各组无显著性变化 ($F = 0.32$; $P > 0.05$)。

结论: 普伐他汀在具有不同致病机制的子痫前期样模型中对临床表现影响不同。