Comparative Study of the Potential Therapeutic Roles of Urocortin-1 and Selective Endothelin Type A Receptor Blockade in Preeclamptic Pregnant Rats (Physiopathological Study)

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Abstract: Administration of urocortin-1 or the selective endothelin type A receptor (ETA) receptor antagonist (ABT-627) might have potential therapeutic effects against preeclampsia. So, this investigation aimed to evaluate and compare the possibility of the use of either urocortin-1 or ETA blockade as a new target of therapeutic approach to preeclampsia. The current study was carried out on 125 female Wistar rats divided into five equal groups. Group I: included virgin non-pregnant rats. Group II: included pregnant rats that were received saline solution (0.5 ml/100 g body weight) from day 7 to day 20 of gestation. Group III: included pregnant rats that were treated with L-NAME dissolved in sterile saline solution in a dose of 10 mg/0.5 ml/100 g body weight subcutaneously and daily starting from the same day of gestation and for the same duration as mentioned for group II. Group IV included pregnant rats that were treated by both L-NAME (the same dose and for the same duration as mentioned for group III) and urocortin-1, in a dose of 5 μg/kg body weight/ day subcutaneously starting from day 14 to day 20 of gestation. Group V included pregnant rats that were treated by both L-NAME (the same dose and for the same duration as mentioned for group III) and ABT-627, 5 mg/kg / day subcutaneously starting from day 14 to day 20 of gestation. Physiological and statistical studies were done. Obtained results revealed that mean arterial blood pressure (MAP) was significantly increased in L-NAME treated pregnant rats, urocortin treated and ABT-627 treated rats on day 13 as compared to controls, normal pregnant rats urocortin and ABT-627 treated rats on day 20, but significant decrease in urine volume was detected in L-NAME treated pregnant rats and urocortin, ABT-627 treated rats on day 13 as compared to controls, none treated pregnant rats. Urocortin treatment caused significant increase in urine volume on day 20 as compared to all other groups. A significant increase in plasma ET-1 was detected in L-NAME treated pregnant rats (on days 13 and 20), Urocortin and ABT-627 treatment caused significant decrease plasma ET-1 on day 20 as compared to L-NAME treated rats on (days 13 and 20) and urocortin treated rats on day 13. Urocortin treatment significantly increased creatinine clearance on day 20 as compared to L-NAME treated pregnant rats (on days 13 and 20), urocortin treated rats (on day 13), and ABT-627 treated rats (on day 13 and 20). Moreover, the pup weigh was increase significantly in urocortin treated rats as compared to L-NAME- treated and ABT-627 treated rats. Conclusion: Preeclampsia could lead to acute kidney injury with physiological alterations in many parameters, meanwhile, urocortin-1 showed ameliorative effect than ET-1 treated rats on all of these parameters. Further research will be needed to study and compare between urocortin-1 and ABT-627 with more pathophysiological parameters.

Keywords: Preeclampsia, Urocortin-1, ABT-627, Placenta, NO.

1. Introduction

During normal pregnancy, important physiological adaptation occurs in the mother that ensures an adequate blood supply to the fetus. Vascular resistance, mean arterial blood pressure (MAP) and sensitivity to endogenous constrictors are reduced, whereas cardiac output, heart rate, and blood volume are increased. This allows the maintenance of placental vasculature in a state of near-maximal dilatation (Greene et al. 2000). Failure to achieve this adaptation may result in the reduced fetoplacental perfusion that develops during preeclampsia (Imperatore et al. 2006).

Preeclampsia (also indexed as pregnancy-induced hypertension, gestosis, and gestational hypertension) is a pregnancy-specific syndrome, recognized from antiquity as a leading cause of maternal and perinatal mortality (Chesley, 1978). It is diagnosed by the accompanying increased blood pressure, proteinuria and edema, all of which appear in the second half of pregnancy. These signs are accompanied by several metabolic disorders. It has been termed the “disease of theories”, reflecting the confusion that surrounds the causes and pathophysiology of pre-eclampsia. Recent insights, however, may be clarifying this enigmatic condition (Pedryez et al. 2005). Aberration of the interaction between placental and maternal tissue is
probably the primary cause, but the exact nature of the differences from normal pregnancy remain elusive.

An important relationship has been demonstrated between nitric oxide (NO) (a potent vasodilator, produced in the vascular endothelium) and blood pressure regulation during pregnancy (Myatt et al. 1993). In pregnant animals chronic inhibition of NO synthesis reverses refractoriness to angiotensin and vasopressin and eventually produces a preeclampsia-like syndrome (Molnar et al. 1994). Several investigators reported the inhibition of NO synthesis with analogues of L-arginine such as N\(^6\)-nitro-L-arginine methyl ester (L-NAME) caused hypertension, proteinuria, fetal growth retardation, and increased fetal mortality without affecting gestational length (Kitamura et al. 1993).

The urocortins are a family of three related peptides, which are members of the CRF family and are expressed mainly in the heart and brain. Urocortins are named for their similar primary structure and bioactivity to both urotensin I and corticotropin-releasing factor (CRF) (Schiffrin, 2005). Human placenta, chorion and amnion express urocortin-1 (Cooke and Davidge 2003). Urocortin-1 levels do not change during pregnancy (Vaughan, 1995). However, maternal plasma urocortin-1 levels were higher at labor, but they did not change significantly throughout the different stages of spontaneous labor (Suda et al. 2004). In explants of human placental tissue at term, the addition of urocortin-1 stimulated prostaglandin E\(_2\) (PGE\(_2\)) release by tissues in a dose dependent manner, and the urocortin-1 and CRF-induced PGE\(_2\) release were not significantly different (Khan and Ng 2004). Starting from the observations that urocortin-1 produces in rats a prolonged hypotensive effect when administered intravenously (Rademaker, 2002), the role for urocortin-1 in intraplacental blood flow regulation was postulated. Human urocortin-1 caused concentration-dependent relaxation of the fetal placental vasculature (Florio et al. 2002).

Endothelin-1 (ET-1), an endothelium-derived peptide, is a potent vasoconstrictor (Florio et al. 2002). It is a 21-amino acid peptide, derived from a 203-amino acid peptide precursor, preproendothelin, which is cleaved after translation to form proendothelin. In the presence of a converting enzyme located in the endothelial cells, proendothelin, or big ET, is cleaved to produce the 21-amino acid peptide ET. Increased synthesis of ET-1 has been reported in various diseases associated with cardiovascular abnormalities, such as hypertension, diabetes, and chronic renal failure (Schringer et al. 2004). ET-1 can elicit either a prohypertensive, antinatriuretic effect by activating ET type A (ETA) receptors, or an antihypertensive, natriuretic effect via ET type B receptor activation. ET-1 receptor binding sites have been identified throughout the body, with the greatest numbers of receptors in the lungs and kidneys (Muramatsu et al. 2001).

ET may play an important role in mediating pathophysiological changes that occur during preeclampsia because endothelial damage is a known stimulus for endothelin synthesis (Karteris et al. 2004). Moreover, elevation of the circulating levels of endothelin in pregnant sheep resulted in a significant increase in mean arterial pressure (MAP), renal vascular resistance, and proteinuria (Granger et al. 2001) all features observed in women with preeclampsia.

Previous studies have reported that long-term elevations in plasma ET-1 concentrations comparable to those measured in women with preeclampsia may play a role in mediating the reductions in renal function and elevations in arterial pressure observed in women with preeclampsia (Granger et al. 2006). Although, other studies have reported no significant changes in circulating ET-1 concentrations during moderate forms of preeclampsia (Greenberg et al. 1997). The importance of locally produced ET in the pathophysiology of preeclampsia remains unclear.

This study aimed to compare between urocortin-1 and the selective ETA receptor antagonist (ABT-627) as adjuvant therapy for preeclampsia using L-NAME treated pregnant rats as an animal model of preeclampsia.

2. Material and Methods

The current study was carried out on 125 female Wistar rats supplied by Medical College animal house at King Khalid University Hospital (King Saud University). Their average weight was 250-300 g. They were 13-18 weeks old. They were housed in a controlled environment and get free access to water ad libitum. Two or three cycling female rats were housed with a male for 24 hours. The presence of sperms in vaginal smears was considered as day 1 of pregnancy. Rats were divided into five groups (25 rats each) according to the following experimental design:

Group I: included virgin non-pregnant rats.
Group II: included pregnant rats that were received saline solution (0.5 ml/100 g body weight) subcutaneously daily and starting from day 7 to day 20 of gestation. Group III: included pregnant rats that were treated with L-NAME dissolved in sterile saline solution in a dose of 10 mg/0.5 ml/100 g body weight subcutaneously and daily starting from the same day of gestation and for the same duration as mentioned for group II, to make an animal model of
pregnancy (Curtis et al. 1995). Group IV: included pregnant rats that were treated by both L-NAME (the same dose and for the same duration as mentioned for group III) and urocortin-1, in a dose of 5 µg/kg body weight/ day subcutaneously starting from day 14 to day 20 of gestation (Davidge et al. 1996). Group V: included pregnant rats that were treated by both L-NAME (the same dose and for the same duration as mentioned for group III) and ABT-627, 5 mg/kg / day (McCarthy et al. 1993) starting from day 14 to day 20 of gestation.

The following parameters were measured in control and all pregnant rats on day 13 of gestation (i.e. 7 days after saline injection in group II or after L-NAME treatment in group III).

1- Mean arterial blood pressure (MAP) from rat-tail by blood pressure system model 1100 HP series based on size-exclusion HPLC (Brinkman et al. 2004).

Blood samples were taken from the eye ball using capillary tubes and collected in EDTA tubes containing aprotinin at 0°C, centrifuged at 1600 g for 15 minutes. Plasma was analyzed for the following:

1- Endothelin-1 (ET-1) using the Parameter ET-1 immunoassay kit, a product from R&D System Inc. USA, Minneapolis, MN. (catalog No. BBE 5) (Suzuki, N et al. 1990).

2- Total plasma nitric oxide products (as the sum of nitrite and nitrate) using the Total Nitric Oxide Assay (catalog No. DE1600), manufactured by R&D System Inc. USA, Minneapolis, MN (Miles et. al. 1996).

3- Angiotensin II (Ag II) using the competitive EIA kits provided from Peninsula Laboratories, Inc. San Carlos, California. Catalog No. S-1133 (EIAH7002) (Volland et. al. 1999).

4- sVCAM-1 using Quatinkine, mouse sVCAM-1 immunoassay (catalog No. MVC00), manufactured by R&D Systems, Inc. USA, Minneapolis, MN (Frijns et. al. 1997).

On day 20 of gestation (i.e. 14 days after saline injection in group II or after L-NAME treatment in group III and 7 days after the start of urocortin-1 or the selective ETA receptor antagonist (ABT-627) administration in groups IV and V, respectively), blood pressure were measured and 24 hrs. urine was collected. The rats were sacrificed and blood was collected via cardiac puncture. Plasma samples were used for the above mentioned parameters. Pup weights were also measured.

**Statistical analysis:**

Results are expressed as mean ± standard deviation (X ± SD). The significance of the difference between the values from different groups is determined using one way analysis of variance (ANOVA) (F-test) combined with Tukey-Kramer Multiple Comparisons Test using GraphPad InStat program. A level of P< 0.05 is defined as statistically significant.

**3. Results**

1. **Mean arterial blood pressure (MAP) (mmHg)**

As shown in table 1, Fig. 1, MAP was significantly increased (P<0.001) in L-NAME treated pregnant rats (on days 13 and 20), urocortin treated rats (on day 13), and ABT-627 treated rats (on days 13 and 20) as compared to controls, normal pregnant rats and urocortin treated rats (on day 20), (134.24±8.575, 154.36±9.878, 149.08±9.678, 154.28±10.163, 119.24±6.234 vs 98.72±6.478, 100.40±7.047, 98.04±8.111, 103.32±5.970, mmHg respectively). This means that urocortin treatment was more effective than ABT-627 treatment in lowering MAP as evidenced by the detected significant changes between the two groups of rats treated by the two drugs separately. Moreover, treatment of rats with ABT-627 significantly lowered the mean ABP on day 20 of gestation (group Vb) as compared to none treated pregnant rats and urocortin or ABT-627 treated rats on day 13 of gestation (groups IIIa, IIIb, IVa, Va).

2. **Urine volume (ml/24 hrs)**

As shown in table 1, Fig. 2, a significant decrease in urine volume was detected in L-NAME treated pregnant rats (on day 13 and 20), and urocortin, ABT-627 treated rats on day 13 as compared to controls, none treated pregnant rats and urocortin treated rats on day 20 (6.516±0.3187, 7.16±0.3484, 6.500±0.379, 7.072±0.237, 6.992±0.473 vs 7.828±0.575, 7.512±0.331, 7.632±0.334, 13.648±0.664 ml/24 hrs, respectively). In addition, a significant increase in urine volume (P<0.0001) was found in urocortin treated rats (on day 20) (13.648±0.664) as compared to all other groups.

3. **Creatinine clearance (ml/min)**

As shown in table 1, urocortin treatment significantly (P<0.0001) increased creatinine clearance on day 20 as compared to L-NAME treated pregnant rats (on days 13 and 20), urocortin treated rats (on day 13), and ABT-627 treated rats (on day 13 and 20) (2.496±0.3470 vs 0.968±0.3211, 0.996±0.4277, 0.888±0.4258, 2.124±0.3431 ml/min respectively) where P<0.0001.
Urocortin treatment significantly (P>0.05) increased creatinine clearance on day 20 as compared ABT-627 treatment. However creatinine clearance was significantly (P<0.001) increased in ABT-627 treated rats on day 20 as compared to L-NAME treated pregnant rats (on days 13 and 20), urocortin and ABT-627 treated rats (on day 13).

4. 24 hrs urinary albumin excretion (mg/dl) (Table 1)

As shown also in table 1, urinary albumin excretion was significantly increased (P<0.001) in all treated pregnant rats whether treated by L-NAME, urocortin or ABT-627 (on days 13 and 20) as compared to controls and normal pregnant rats (on days 13 and 20) (130.24±7.149, 130.88±7.026, 130.72±4.505, 59.92±6.582, 128.96±5.319, 72.88±8.589 vs 27.80±1.958, 26.12±2.261, 27.24±2.350 mg/dl, respectively). But urinary albumin excretion was significantly (P<0.001) decreased in urocortin treated rats (on day 20) as compared to L-NAME treated rats (on days 13 and 20) and urocortin treated rats (on day 13) and ABT-627 treated rats (on day 13 and 20). Moreover significant (P<0.001) decrease was detected in ABT-627 treated rats (on day 20) as compared to L-NAME treated rats (on days 13 and 20) and urocortin and ABT-627 treated rats on day 13.

5. Pup weight (g)

As shown in table 1, Fig. 3, pup weight was significantly decreased (P<0.001) in L-NAME treated pregnant rats as compared to normal pregnant rats, urocortin treated rats (1.09±0.3600 vs 2.41±0.77519, 1.71±0.3590 g respectively). Also pup weight was significantly (P<0.001) decreased in urocortin and ABT-627 treated rats as compared to normal pregnant rats. While significant increase was detected in urocortin treated rats as compared to ABT-627 treated rats.

6. Plasma endothelin-1 (ET-1) (pg/ml)

As shown in table 1, Fig. 4, a significant (P<0.001) increase in plasma ET-1 was detected in L-NAME treated pregnant rats (on days 13 and 20), urocortin treated rats, ABT-627 treated rats (on days 13) as compared to controls and normal pregnant rats (on days 13 and 20) (0.53±0.04776, 0.558±0.05276, 0.548±0.06245, 0.520±0.04957 vs 0.410±0.05243, 0.447±0.05587, 0.442±0.05292, pg/ml respectively). Urocortin and ABT-627 treatment caused significant (P<0.001) decrease plasma ET-1 on day 20 as compared to L-NAME treated rats on (days 13 and 20) and urocortin treated rats on day 13. In addition ABT-627 treated rats on day 13 showed significant increase as compared to the same group on day 20.

7. Total serum nitric oxide (NO) products (µmol/l)

As shown in table 1, Fig. 5, a significant decrease (P<0.001) in total serum NO products (nitrite + nitrate) was detected in L-NAME treated pregnant rats (on days 13 and 20), urocortin treated rats (on day 13), and ABT-627 treated rats (on days 13) as compared to controls and normal pregnant rats (on days 13 and 20), urocortin treated rats (on day 20) and ABT-627 treated rats (on day 20) where the mean values were 161.01±13.055, 126.14±13.351, 161.30±12.260, 158.97±13.973 vs 245.48±16.858, 247.74±18.956, 249.83±16.474, 250.78±20.318, 250.63±21.551 µmol/l respectively. Moreover L-NAME treated rats on day 20 showed significant decrease as compared to L-NAME, urocortin and ABT-627 treated rats on day 13.

8. Plasma angiotensin II (Ag II) (ng/ml)

As shown also in table 1, Fig. 6, plasma Ag II was increased significantly (P<0.001) in L-NAME treated pregnant rats (on days 13 and 20), urocortin treated rats (on days 13), and ABT-627 treated rats (on days 13 and 20) as compared to controls, normal pregnant rats (on days 13 and 20) and urocortin treated rats (on days 20) (2.06±0.4031, 2.128±0.4496, 2.116±0.3625, 2.224±0.4275, 1.712±0.2948, vs 1.000±0.3391, 1.088±0.3866, 0.912±0.3655, 1.192±0.3451 ng/ml respectively). Moreover significant (P<0.001) decrease was found in urocortin treated rats (on day 20) as compared to the same group of rats and ABT-627 treated rats but on day 13 and the normal pregnant rats (on days 13 and 20) rats.

9. Serum VCAM-1 (ng/ml)

As shown in table 1, Fig. 7, a significant increase (P<0.001) in serum VCAM-1 was detected in L-NAME treated rats (on days 13 and 20), urocortin treated rats (on day 13), and ABT-627 treated rats (on day 13 and 20) as compared to controls and normal pregnant rats (on days 13 and 20) where the mean values were 35.96±7.120, 45.59±6.209, 35.95±8.151, 35.22±10.048, 25.38±6.091 vs 19.44±5.122, 18.72±5.237, 17.87±4.972 ng/ml respectively. Moreover significant (P<0.001) increase was detected in L-NAME treated rats (on day 20) as compared to L-NAME treated rats (on day 13) and urocortin treated rats (on day 13 and 20), ABT-627 treated rats (on day 13 and 20). While urocortin and ABT treatment caused significant decrease of serum VCAM-1 on day 20 as compared to L-NAME, urocortin, ABT-627 treated rats on day 13.
Table I: Mean values ±SD of the measures parameters in control rats, none treated and treated pregnant rats.

<table>
<thead>
<tr>
<th></th>
<th>None treated</th>
<th>L-NAME-treated</th>
<th>Urocortin-treated</th>
<th>ABT-627-treated</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group IIa (13)</td>
<td>Group IIb (20)</td>
<td>Group IIIa (13)</td>
<td>Group IIIb (20)</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>98.72±9.48</td>
<td>100.4±9.05</td>
<td>104.8±8.11</td>
<td>134.2±8.38</td>
<td>154.3±9.88</td>
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<tr>
<td>Urine volume (ml/24 hr)</td>
<td>7.82±0.575</td>
<td>7.51±0.331</td>
<td>7.63±0.334</td>
<td>6.51±0.218</td>
<td>6.50±0.379</td>
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<tr>
<td>Creatinine clearance (ml/min)</td>
<td>2.64±0.0379</td>
<td>2.62±0.4233</td>
<td>2.64±0.2551</td>
<td>0.96±0.3145</td>
<td>0.86±0.3211</td>
</tr>
<tr>
<td>Urinary albumin excretion (mg/dl)</td>
<td>27.8±1.958</td>
<td>26.1±2.261</td>
<td>27.2±2.350</td>
<td>130.2±7.149</td>
<td>130.8±7.026</td>
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<tr>
<td>Pup weight (g)</td>
<td>2.4±0.7519</td>
<td>1.09±0.3600</td>
<td>1.71±0.3590</td>
<td>1.22±0.3803</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plasma endothelin-1 (pg/ml)</td>
<td>0.41±0.0524</td>
<td>0.44±0.0595</td>
<td>0.44±0.0529</td>
<td>0.53±0.0473</td>
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<tr>
<td>Serum NO products (µmol/l)</td>
<td>245.4±16.86</td>
<td>247.7±18.96</td>
<td>249.8±16.47</td>
<td>161.0±15.06</td>
<td>126.1±13.53</td>
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<td>Ag II (ng/ml)</td>
<td>1±0.0391</td>
<td>1.0±0.3664</td>
<td>0.91±0.3655</td>
<td>2.06±0.4031</td>
<td>2.12±0.4496</td>
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<tr>
<td>VCAM-1 (ng/ml)</td>
<td>19.4±5.122</td>
<td>18.7±5.237</td>
<td>17.8±4.972</td>
<td>35.9±7.120</td>
<td>45.5±7.209</td>
</tr>
</tbody>
</table>

* Significant changes at P<0.0001

Figure 1: Mean values ± SD of the mean arterial blood pressure (mmHg) in the studied groups.

Figure 2: Mean values ± SD of urine volume (ml/24 h) in the studied groups.
Figure 3: Mean values ± SD of pup weight (g) in the studied groups.

Figure 4: Mean values ± SD of plasma endothelin-1 (pg/ml) in the studied groups.

Figure 5: Mean values ± SD of total serum NO products (µmol/l) in the studied groups.

Figure 6: Mean values ± SD of plasma angiotensin II (ng/ml) in the studied groups.

Figure 7: Mean values ± SD of serum VCAM-1 (ng/ml) in the studied groups.
4. Discussion

Preeclampsia is a leading cause of maternal and perinatal morbidity and mortality. Although the precise cause is unknown, endothelial cell dysfunction and inflammation are important aspects of the overall puzzle (Redman et al. 1999). Ness and Roberts (1996) have hypothesized that there are both placental and maternal origins of preeclampsia. The maternal origins could be preexisting maternal disorders often not evident before pregnancy, which predispose to preeclampsia by endothelial dysfunction and which later predispose to cardiovascular disease. According to the above cited data the overall goal of the present study is to assess the role of urocortin and ABT-627 treatment on renal function and production of ET-1, total NO products, Ag II, and VCAM-1 in L-NAME treated pregnant rats as a model of preeclampsia. For our knowledge, no previous studies have been carried out on the comparison between the beneficial effects of ABT-627 and urocortin-1 against L-NAME induced preeclampsia. So, we can through more light to find out which one of them more able to reduce the physiopathological changes caused by L-NAME treatment as a model of preeclampsia.

In this study we used an animal model of preeclampsia by using L-NAME, a nitric oxide synthase (NOS) inhibitor. Several investigators found that L-NAME treated pregnant rats show preeclampsia-like symptoms consisting of a dose-dependent hypertension maintained through to term. The chronically NOS-inhibited pregnant rat also exhibited renal vasoconstriction leading to a decrease in GFR, proteinuria, suppression of the normal volume expansion, and increased maternal and fetal morbidity and mortality in a pattern that resembled preeclampsia (Halligan et al. 1994). All of these effects are attributed to the known vasoconstrictor effect of L-NAME. These findings are remarkably similar to those seen in human preeclampsia and support the hypothesis that a decrease in the bioavailability of NO might thus play a role in the development of the disease (Ma et. al. 1999). In the present study we found a significant decrease in serum NO levels in pregnant rats treated with L-NAME both on day 13 and 20 of gestation. Both urocortin and ABT-627 significantly reduced total NO products on day 20 as compared to none treated preeclamptic rats. But no significant change in total NO products was detected between urocortin and ABT-627 treated rats (on day 20) as compared to each other, to controls or to normal pregnant rats (on days 13 and 20). Data on the production of NO in preeclampsia are controversial. There are reports that demonstrated plasma NO levels to be reduced (Seligman et. al. 1994), unchanged (Silver et al. 1996), or elevated (Smarason et. al. 1997).

In the current study, L-NAME treated pregnant rats (whether on day 13 or 20 of gestation) showed significant rise of both MAP and 24 hrs urinary albumin excretion together with significant reduction of each of creatinine clearance, urine volume and pup weight as compared to pregnant rats receiving saline solution. These findings are consistent with the previous reports (Wang et. al. 2000). Urocorotin treatment from day 14 to 20 of gestation significantly decreased MAP as compared to none treated rats, while ABT-627 treatment for the same period of gestation did not significantly decreased MAP. This vasodilator effect could be attributed to increased serum NO level or decreased plasma Ag II level as evident in the present study. Moreover, urocortin treatment produced powerful diuretic effect as compared to ABT-627 treatment which also could contribute to its more powerful hypotensive effect. In sheep with experimental heart failure, intravenous infusion of urocortin decreased MAP (Rademaker, 2002). In the same condition, urocortin decreased plasma renin activity, and ET-1, and produced dose-dependent, sustained increases in urine volume, sodium excretion, and creatinine clearance (Rademaker, 2002).

ABT-627 treatment has no significant effect on creatinine clearance in contrast urocortin treatment which significantly increased creatinine clearance as compared to none treated preeclamptic rats. Concerning 24 hrs urinary albumin excretion, both urocortin and ABT-627 treatment could significantly reduce it as compared to none preeclamptic treated rats. The increase in pup weight was more significant by urocortin treatment as compared to ABT-627 treatment. The previous result may explain the decreased pup weight in L-NAME treated rats due to the disturbance in protein synthesis caused by ET-1 mRNA expression (Battistini et. al. 1993). While Fiore et. al. (2005) implicated ET-1 as a causative factor for oxidative stress in preeclampsia by altering the balance between oxidant and antioxidant forces in favor of oxidation. The reduced pup weight in our investigation could be also due to the increased MAP induced by increased production of ET-1 in none treated preeclamptic rats.

The improvement of the previous parameters in urocortin treated rats could be explained according to Abdelrahman et. al. (2005), when urocortin given intravenously in animal models and was found a vasodilatory via arteriole and cardiac CRF2 receptors. Conversely, CRF2-deficient mice exhibit elevated MAP suggesting an endogenous relaxant function of urocortin/CRF2 interactions on vasculature in vivo (Atamer et. al. 2005). The vasodilatory effects of urocortin in rat thoracic aorta are mediated by protein
kinase A and MAP kinase signaling pathways (Kageyama et al. 2003), and relaxation of pulmonary arteries involves inhibition of a protein kinase C-dependent contractile mechanism (Chan et al. 2004). Vasodopressor effects of urocortin in rodents did not appear to involve activation of the nitric oxide/L-arginine pathway, prostanoid production, or K+ channels and were not counteracted by compensatory vasoconstrictive mechanisms (e.g., angiotensin, endothelin) (Gardiner et al. 2005).

ET-1 is a potent endogenous vasoconstrictor peptide that participates in the regulation of vascular tone. Plasma ET-1 concentrations are increased in women with preeclampsia as demonstrated by Baksu et al. (2005). In conjunction with decreased production of prostacyclin and NO, ET-1 could contribute to the hypertension that characterizes preeclampsia (Aydin et al. 2004). Our study demonstrated elevated ET-1 levels in L-NAME treated pregnant rats on days 13 and 20 of gestation as compared with control rats. These results are consistent with the study of Vural, 2002, who reported significant increase in plasma ET-1 level in preeclamptic women in comparison with both nonpregnant and normotensive pregnant women. The mechanism of the ET-1 increase in preeclampsia is unknown, but some hypotheses can be made (Napolitano et al. 2000). First, ET-1 is basically local factor acting at the junction between the endothelium and the vascular smooth muscle layer. The disruption and destruction of these anatomical boundaries can lead to a leak of ET from its local environment to the circulation with subsequently higher peripheral blood levels. Second, an abnormal production of ET by the affected endothelium might be a source for its increase, both locally and in the peripheral blood. Third, the increased production of ET-1 from the placental or fetal tissue in preeclamptic pregnancies or increased diffusion into the maternal circulation might explain the increased levels found in preeclampsia. In fact, increased trophoblastic ET-1 mRNA expression in preeclamptic pregnant versus normal pregnant women was found (Napolitano et al. 2000). Both urocortin and ABT-627 treatment could significantly reduce ET-1 level as compared to L-NAME treated rats on (days 13 and 20).

In the present study plasma Ag II was significantly increased in L-NAME treated pregnant rats (on days 13 and 20), urocortin treated rats (on days 13), and ABT-627 treated rats (on days 13 and 20) as compared to controls, normal pregnant rats (on days 13 and 20) and urocortin treated rats (on days 20). This means that urocortin treatment significantly reduced Ag II level as compared to ABT-627 treatment. Recently novel angiotensin II-related biomolecular mechanisms have described in preeclampsia and may explain the primary clinico-pathologic features of preeclampsia (Shah, 2006). Yang et al. 2006 investigated the relationship between urocortin and the activity of angiotensin-converting enzyme (ACE), which plays a key role in producing the potent vasoconstrictor angiotensin II (Ag II). Urocortin was acutely and subchronically administered to rats and then the serum ACE level was evaluated. They found that on prolonged administration of urocortin, the serum ACE level remained low. These findings support the changes in MAP following administration of urocortin in the present study. Thus, the changes of the ACE activity and its effect on production of Ag II may play a role in the vasodilatory property of urocortin.

Urocortin and ABT-627 treatment caused significant decrease VCAM-1 level on day 20 as compared to L-NAME treated rats on days 13 and 20. Greer et al. 1994 showed that soluble VCAM-1 was elevated in the serum of preeclamptic patients. They demonstrated that increased cytokine levels were closely correlated with elevated levels of VCAM-1. Visser et al. 2002 reported that plasma concentration of sVCAM-1 reflect the release of TNF-α and provide sensitive marker of the excessive release of this cytokine in preeclampsia. A significant increase in serum sVCAM-1 was detected in L-NAME treated rats (on days 13 and 20), urocortin treated rats (on day 13), and ABT-627 treated rats (on day 13 and 20) as compared to controls and normal pregnant rats (on days 13 and 20). Urocortin treatment significantly reduced sVCAM-1 level on day 20 of gestation as compared to none treated rats on the same day. Increased circulating levels of sVCAM-1 may be a result of increased shedding from activated endothelial cells which express VCAM-1 upon activation by several cytokines as TNF-α (Caivoropongsa et al. 2002). Moreover, VCAM-1 is also induced by oxidatively modified lipoprotein, which has been implicated in the pathogenesis and oxidative stress of preeclampsia (Zeisler et al. 2001). Another possible mechanism for increased sVCAM-1 concentration is that of leukocyte activation, which has been implicated in the endothelial dysfunction and organ damage observed in preeclampsia (Wilezynski et al. 2002). The biological role of sVCAM-1 is, as yet, unresolved. Release of these surface molecules may function to break adhesive interaction between cells, and may assist regulation of the inflammatory process. Nevertheless, they may have an important role in the attachment and transendothelial migration of leukocytes, angiogenesis, endothelial activation
and interaction in the unregulated inflammatory process, which characterizes preeclampsia (Coata et al. 2002).

In view of these findings, it is concluded that administration of L-NAME induced functional renal changes. Urocortin-1 or ABT-627 administration to NOS deprived pregnant rats as an animal model of preeclampsia has powerful vasodilatation/hypotensive actions and renoprotective effect. Urocortin-1 ameliorated these changes strongly than ABT-627. Thus urocortin-1 was important for prevention of preeclampsia induced nephrotoxicity. Further investigation for exploring its beneficial effect and its usefulness in human nephrotoxicity is recommended.

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