Hemodynamic and Renal Effects of Bosentan and Losartan in Endothelin-1 And Angiotensin II Induced Hypertensive Rats

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1. Introduction

Endothelin-1 is the most potent known vasoconstrictor. It is first discovered by (Yanagisawa et al., 1988). Beside ET-1, other vasoconstrictors have great roles in cardiovascular diseases one of them is Ang II. It is one of the main substances in the renin-angiotensin-system (RAS), produced from angiotensin I (Ang I) by action of angiotensin converting enzyme (ACE). ET-1 has multiple physiological roles in body organs through widely distribution of ET-1 receptors over tissues (Stow et al., 2011). The most common ET-1 function is a potent vasoconstrictor in most vascular beds (Potts et al., 2013).

In addition to growth promoting and vasoconstriction properties of ET-1, it has a central role in the pathogenesis of proteinuria and glomerulosclerosis in the kidney, which is mediated via activation of the ETA receptor that leads to natriuresis and diuresis action (Kohan, 2010; Kohan et al., 2011). (Verhaar et al., 1998) demonstrated that ET-1 binds with ETB receptor and increases NO and prostacyclin, for this reason, administration of ET-1 intravenously has a biphasic response. In contrast, NO inhibits the ET-1 expression also NO antagonizes ET-1 by lowering the concentration of ET-1 than required elicit vasodilation (Khimji and Rockey, 2010).

Because most of the physiological actions of Ang II occur via AT1 receptor, so that blocking AT1 is considered more significant than AT2 receptors like ACEls, angiotensin II receptor blockers decrease blood
pressure but not affect heart rate (Sookoian et al., 2005). Although, Ang II stimulates adrenal cortex aldosterone, which causes elevated Na+ and water retention, then, it leads to volume load (Knights et al., 2006), and the result is elevation of SBP. There is few research about the relation of ET-1 and Ang II actions, therefore, this work was designed to study how bosentan affect Ang II and how losartan is related with ET-1 actions in induced hypertensive rats. So, the aim of the present study is to investigate the short term hemodynamic and renal effects of bosentan and losartan in ET-1 and Ang II infused rats.

Materials and Methods

Animals

Forty-eight male rats weights between 300 – 400 grams were used, overnight fasted (8-12 hrs.) and animals were brought to the laboratory at the day of surgery. Six rats were kept for each plastic cage. The employed experimental animals were met the criteria of ethic rules of the supervising committee of College of Science. The animals were anesthetized by intraperitonial infusion of combination Ketamine hydrochloride 80 mg/Kg (Trittau, Germany) and Xylasin 12mg/Kg (Interchem, Holland). The depth of anesthesia was monitored by loosing reflex. The anesthesia was remained for 1.5 – 2 hours and a supplement dose was used if necessary. Rat’s body temperature controlled between 35-37 oC.

Cannulation of femoral vein for intravenous infusion

Small incision (2-3 cm) was made on the right thigh using concave sterile scissors, outer layer of skin was removed and the matrix of collagen fibers interlaced with elastic fibers of the dermis and cleaned carefully, then a 27 G ½ needle filled with heparinized normal saline 10 IU/ml was inserted into the vessel which is attached to the polythene tube (ID 0.58 mm, OD 0.96 mm. England) that was connected to infusion pump (Advance series 1200 infusion system, USA) through syringe 10 ml size.

Immediately after insertion of cannula the normal saline was infused.

Procedure for cannulation of carotid artery

The common carotid arteries and two vagus nerves run together on either side (right and left) of trachea. Carotid artery had been raised, back head of forceps placed under it, the cephalic end of artery was tied with a ligature to prevent blood flow toward the brain and Bulldog (Larca, Germany) was clamped cardiac end of the carotid artery. A small cut was made near cephalic end by fine scissor (Eye max, Germany) through this fine rupture a polythene tube (ID 0.58 mm, OD 0.96 mm. England) filled with heparinized normal saline (0.9% sodium chloride) and 10 IU/ml was inserted gently, pulled toward cardiac end till reach Bulldog, a thread loosely tied around them and then clamp slowly released.

Recording invasive blood pressure and heart rate

Direct blood pressure was recorded through cannulated right carotid artery. For this purpose, the cannulated artery had been attached to three-way stopcock connected to the pressure transducer (Memscap SP 844, Norway) and a syringe (Ayset 10ml) filled with heparinized saline. Software was designed (Lab Chart 6, Blood pressure module, ADInstrument Australia) were analyzed those signals into mean arterial pressure and heart rate.

Experimental design

Two experiments were carried out. In experiment I, hypertensive rats were developed by ET-1 infusion. The experimental rats were divided into two groups. In the first group, animals were infused 15 ml/Kg/h and the BP was monitored each 10 minutes until sixty minutes. The rats from the second group were subdivided into three subgroups, each subgroup includes six rats. The first subgroup served as a positive control (Animals were infused by ET-1, 520 ng/min/Kg). The second subgroup were injected with bolus infusion of bosentan 10 mg/0.5 ml/Kg while, the last subgroup was
infused bolus infusion of losartan 10 mg/0.5ml/Kg. The same design was done in experiment II, but the model of hypertension was developed by Ang II infusion (320 ng/min/Kg).

**Determination of serum Ca2+**

Calcium in all specimens was determined spectrophotometrically using BioLabo kits, France.

**Determination of electrolytes excretion rates**

Excretion rates were found by following equations:

\[
\text{Sodium excretion rate (mEq/hr/Kg)} = \text{Urine flow (ml/hr/Kg b.w)} \times \text{Na+ concentration in Urine mEq/L}
\]

\[
\text{Potassium excretion rate (mEq/hr/Kg)} = \text{Urine flow (ml/hr/Kg b.w)} \times \text{K+ concentration in Urine mEq/L}
\]

**Determination of serum NO**

This determination relied on Griess reagent system which is based on the chemical reaction, using sulfanilamide and N-1-napthylethlenediamine dihydrochloride (NED) under acidic (phosphoric acid) conditions.

**Determination of Packed cell volume (PCV)**

Three quarters of the heparinized capillary tubes were filled with whole blood and closed by commercial clay then centrifuged by micro-hematocrit centrifuge (Hawksley, England) for 5 minutes at 1000g. The PCV was read using microhaematocrit reader.

**Statistical analysis**

All data were expressed as means ± standard error (SE) and statistical analysis was carried out using available statistical software (statistical package for social science (SPSS) version 16). Data analysis was made using one way analysis of variance (ANOVA). The comparisons among groups were done using Duncan post hoc test. P values <0.05 were considered as significant.

**Results**

Statistical analysis revealed that, MAP was significantly increased (P<0.05) in ET-1 infused rats after thirty, fifty and sixty minutes compared to the saline infusion (Figure 1, A). On the other hand, it was raised significantly when Ang II infused but immediately after only 10 minutes of infusion. Bolus infusion of bosentan caused a slightly decrease in MAP, whereas losartan infusion significantly decreased MAP comparing to ET-1 infused rats (Figure1, A). Furthermore, (Figure 2, A) shows that MAP tended to reduce significantly (P<0.01) at thirty minute time interval when losartan injected in Ang II infused rats, while bosentan infusion did not reduce MAP significantly. Neither bosentan nor losartan significantly changed H.R in ET-1 infused rats as compared with saline group (Figure 1,B), while Ang II infusion at 20 minutes
significantly decreased it when compared with control group. However, both ET-1 and Ang II receptor antagonists did not alter H.R significantly (Figure 2, B).

**Figure 1:** Effects of bosentan and losartan on mean arterial blood pressure (MAP), A) and heart rate H.R. B) in ET-1 induced hypertensive rats.

Saline (15ml/kg/h) was infused, Endothelin-1 (ET-1) (520 ng/min/Kg) was infused for one hour, bolus infusion losartan (10 mg/Kg) immediately Endothelin-1 (520 ng/min/Kg was infused for one hour, and bolus infusion bosentan (10 mg/Kg) then Endothelin-1 (520 ng/min/Kg) was infused for one hour. Data are expressed mean ± standard error (mean ± SEM). #P<0.05 vs saline group and *P<0.05 vs Endothelin-1 group. One way ANOVA and post-hoc Duncan were used.

Table 1 and 2, shows that serum NO level in ET-1 and Ang II induced hypertensive rats slightly decreased but not significantly (P>0.05) as compared with saline group. Neither losartan nor bosentan significantly increased serum NO level as compared with ET-1 or Ang II infused rats. PCV value was markedly increased (P<0.05) in ET-1 group, whereas in bosentan and losartan groups it was slightly decreased as compared with ET-1 infused rats (Table 1). Serum calcium concentration significantly (P<0.01) elevated in ET-1 induced hypertensive rats as compared with saline group (Table 1).

**Figure 2:** Effects of bosentan and losartan on mean arterial blood pressure (MAP), A) and heart rate (H.R), B) in Ang II induced hypertensive rats.

Saline (15ml/h/kg) was infused, Angiotensin II (Ang II) (320 ng/min/kg) was infused for one hour, bolus infusion losartan (10 mg/Kg) immediately Ang II (320 ng/min/Kg) was infused for one hour, and bolus infusion bosentan (10 mg/Kg) then Ang II (320 ng/min/Kg) was infused for one hour. Data are expressed mean ± standard error (mean ± SEM). #P<0.05 vs saline group and *P<0.05 vs Ang II groups. One way ANOVA and post-hoc Duncan were used.

**Table 1:** Effects of bosentan and losartan on serum NO, PCV percentage, and serum Ca2+ in ET-1 induced hypertensive rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NO</th>
<th>PCV</th>
<th>Ca2+</th>
</tr>
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<tbody>
<tr>
<td>Group</td>
<td>µmole/L</td>
<td>(%)</td>
<td>(mg/dL)</td>
</tr>
<tr>
<td>Saline</td>
<td>15.50</td>
<td>45.40</td>
<td>8.791</td>
</tr>
<tr>
<td></td>
<td>± 2.069</td>
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</tr>
<tr>
<td>ET-1</td>
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<td>ET-1 + Bosentan</td>
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<td>± 1.701</td>
<td>± 0.557</td>
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<td>ET-1 + Losartan</td>
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<td>47.00</td>
<td>9.804</td>
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<tr>
<td></td>
<td>± 0.757</td>
<td>± 2.073</td>
<td>± 0.245</td>
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NO, nitric oxide; Ca2+, Calcium; PCV, Packed cell volume; Data expressed as (Mean ± SE) and ANOVA was used for analysis post-hock Duncan. * P<0.05 vs saline. ** P<0.01 vs saline.

**Table 2:** Effects of bosentan and losartan on serum NO, PCV, and serum Ca2+ concentration in Ang II induced hypertension rats

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Figure 3 A, shows that sodium excretion rate tended to increase significantly (P<0.05) in losartan group at twenty minutes and in bosentan group in both twenty and sixty minutes as compared with ET-1 group. Furthermore, potassium excretion rate significantly (P<0.05) increased at twenty minutes in losartan group and at sixty minutes in bosentan group as compared with ET-1 infused rats (Figure 3, B). On the other hand, Ang II infusion caused a significant increase in K+ excretion rate at sixty minutes as compared with saline group (Figure 4, B). In contrast to bosentan administration which caused a slight decrease in K+ excretion rate, bolus infusion of losartan significantly (P<0.05) elevated it as compared with Ang II infused rats.

Figure 4: Effects of bosentan and losartan on sodium (A) and potassium(B) excretion rates in Ang II induced hypertensive rats.

Saline, rats were infused (15ml/h/Kg); Ang II, Angiotensin II infusion (320 ng/min/Kg); Ang II + Bosentan, bolus bosentan (10 mg/Kg) was infused then Ang II infusion (320 ng/min/Kg); Ang II + Losartan, bolus Losartan was infused then Angiotensin II infusion (320 ng/min/Kg). Data expressed as (Mean ± SE) and ANOVA was used for analysis post-hock Duncan. * P<0.05.

Discussion

Hypertension was induced by infusion of ET-1 or Ang II in two different sub experiments to investigate the therapeutic impacts effects of bosentan and losartan on hemodynamic and renal parameters. There is now evidence that, ET-1 enhances free radical production (Viel et al., 2008) and such oxidative stress increases blood pressure (Rodrigo et al., 2007). Another possible mechanism for elevating blood pressure is that ET-1 infusion ought to inhibit of natriuresis as reduced Na+ and K+ excretion rate (Figure 3, A and B). However, the action ET-1 on hypertension is still controversial, but our experiment by infusion ET-1 explained some hemodynamic effects. For instance,
infusion of ET-1 for sixty minutes caused rising MBP. For explaining that, several mechanisms may contributes to ET-1 through ETA blood vessels constriction which caused an increases in peripheral vascular resistance and then increased BP. ET-1 induced vasoconstriction after binding to ETA receptors occurs via G protein-dependent signal transduction pathway of phospholipase C activation second messengers Inositol triphosphate (IP3) and diacylglycerol (DAG) and the IP3 releases Ca2+ from endoplasmic reticulum (Xuan and Glass, 1996).

Thus, ET-1 has direct inotropic and chronotropic effects on heart (Sakai et al., 1999), thereby increases blood pressure. It was cleared that, ET-1 increases neurohormonal active reflex and increase erythropoietin, then BP pressure tended to increase (Kohan et al., 2011). Because ET-1 acts in different pathways, it requires enough time to elevate blood pressure, especially MAP (Figure 1, A). Bolus pre infusion of bosentan and losartan could decrease BP, but only losartan reached the level of significance (P<0.05). The only possible mechanism to illustrate that, ET-1 has strong positive feedback on Ang II (Yoshida et al., 1992). Therefore, we supposed that the short-term effect of rising blood pressure by ET-1, might enhancing Ang II activity. Therefore, blocking Ang II receptors would diminish the actions of ET-1. Another possible mechanism of weak hypotensive effect of bosentan might to block both ETA and ETB which could not increase serum NO level (Table 1).

In experiment II, intravenous infusion of Ang II continuously for one hour caused increases MAP from ten minutes after infusion until the end of the experiment, but the rising in BP was not equal in all time intervals. Ang II activation AT1 receptor causes constriction in blood vessels, and increase peripheral resistance that leads to elevation of BP. Therefore, blocking AT1 by pre infusion of losartan could decrease MAP. Also, Ang II by direct positive inotropic and chronotropic effects on heart (Modgil et al., 2012). However, Ang II stimulates adrenal cortex aldosterone, which causes elevated Na+ and water retention, then, it cause to volume load (Knights et al., 2006) and the result is elevation of BP. Statistical analysis revealed that significant elevated MAP was observed at the beginning then it reduced at the end of the experiment. The possible mechanism for this finding may be due to the pressure-natriuresis through ET-1 A receptor (Kittikulsuth et al., 2011) and pressure-diuresis mechanism (Kohan et al., 2011) showed that even small increases in arterial pressure often causes marked increase in urinary excretion of sodium and water. Interestingly, losartan rather than bosentan reduces BP even though ET-1 had been infused for increasing BP. In Ang II induced hypertension, bosentan also had no role in reducing BP. Serum NO acts against ET-1 effects through dilating blood vessels and decreases the elevated BP (Khimji and Rockey, 2010). The present results showed that intravenous infusion of ET-1 could decrease slightly serum NO (Table 1). Also, both bosentan and losartan increased serum NO level. The possible reason of this reduction of serum NO level may be through NOS inhibition. However, ET-1 receptor elevate NO production through its ETB (Stow et al., 2011), but the reduction in our results may be due to the action of ETA receptor (Verhaar et al., 1998). The physiological elevation of NO levels by losartan bolus infusion may also be due to the reduction of ET-1 and Ang II actions as they have roles for reducing NO level.

For the first time, we showed that intravenous infusion of ET-1 for one hour, could increase PCV significantly (P<0.05), and at the same time bolus pre infusion, either bosentan or losartan could slightly decrease PCV. The possible explained mechanism is that, ET-1 decreases pressure natriuresis by constricting nephrons and increases Bowman’s hydrostatic pressure and then decreases GFR (Elmarakby et al., 2003). The second possible mechanism of PCV reduction after ET-1 infusion by inhibition Na+-K+ ATPase through ETB receptor, thereby more water enters the RBC lead to elevation PCV (Prasanna et al., 2001).
However, previous studies explained that ET-1 decrease serum Ca2+ through parathyroid hormone (PTH) inhibition (Chang et al., 2000), but the rational explanation for increasing serum Ca2+ by short term ET-1 infusion is that ET-1 directly causes the influx of Ca2+ into the cell through L-type calcium channel via IP3 mediation (Bkaily et al., 2011). In contrast to previous studies of natriuretic effects of ET-1 (Bugaj et al., 2008), the present result showed that intravenous systemic infusion of ET-1 caused antinatriuretic effects, because it decreased Na+ excretion rate in ET-1 compared to saline group. (Nakano and Pollock, 2009) demonstrated that ET-1 has natriuretic effect in female rats but not in male rats, and the present study conducted on male, therefore the decrease of urine concentration and excretion might be due to sex difference.

In the present result bosentan could increase Na+ excretion rate, because of removing of ET-1 effects on renal blood vessels. However, the exact relation between ET-1 and K+ homeostasis is not well explained, but the possible mechanism of reduction in K+ excretion rate by ET-1 infusion is stimulation of 20 hydroxyeicosatetraenoic acid (20-HETE) formation, which in turn can inhibit Na+-K+ ATPase activity (Escalante et al., 2002) and consequently K+ excretion may be reduced. Ang II has mutual signaling pathways with NO, Ang II via activation of AT2 lead to NO production in the endothelial cells (Yan et al., 2003). While, the present result demonstrated that continuous infusion of Ang II decreases serum NO slightly. Ang II through AT1 receptor constricted blood vessels then decrease the serum NO concentration and lead to BP elevation and then endothelial cell function diminished.

At the first 20 minutes of Ang II infusion, Na+ excretion rate tended to increase. The pressure natriuresis mechanism may be the possible reason behind the present finding. The present finding of Ang II inducing natriuresis is similar to ET-1 effects. Because both them increases BP and consequently elevated Na+ excretion. The depressant effects of Ang II on potassium excretion rate may be due to decreasing GFR and reducing urine excretion rates (Quadri, 2013). This is clearly observed in the current result (Figure 4, B). The present result is also consistent with the finding of (Cho et al., 2012), who showed that, Ang II increases sodium-2 chloride- potassium co transporter mechanism, so that potassium reabsorption increased and then urine potassium excretion decreased.

In conclusion, intravenous infusion of Ang II immediately (after 5 minutes) elevated MAP, produced upstroke, while MAP tended to elevate after twenty minutes of ET-1 infusion. ET-1 worked in several pathways to perform hypertensive action, it mostly decrease serum NO level. Interestingly, losartan rather than bosentan reduces BP even ET-1 had been infused for increasing BP. In contrast to previous studies of natriuretic effects of ET-1, the present result showed that intravenous systemic infusion of ET-1 caused antinatriuretic effects, because it could decrease Na+ excretion rate in ET-1 compared to saline group. In contrast to bosentan administration, which caused a slight decrease in K+ excretion rate, bolus infusion of losartan significantly elevated it as compared with Ang II infused rats.

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References


