Effect of Bosentan and Losartan on Oxidative Stress and Cortisol levels in Endothelin-1 and Angiotensin II Treated Rats

Sarkawt Hamad Ameen Hamad[1*]; Ismail Mustafa Maulood[2]; Almas MR Mahmud[3]

[1]Biology Department, Faculty of Science, Soran University, Soran, Iraq; [2 and 3]Biology Department, College of Science, Salahaddin University-Erbil, Erbil,Iraq
*Corresponding author: sarkawt.hamad@soran.edu.iq

Abstract: The exact mechanism by which Endothelin-1 (ET-1) and Angiotensin II (Ang II), and their antagonists act in physiology are controversial subjects among researchers. Therefore, the present study aimed to determine the effects of bosentan and losartan on oxidative stress and serum cortisol levels. The present design included two experiments. The first one contains four groups: Group 1 saline infusion, Group 2, ET-1 infusion, Group 3, Bosentan + ET-1, and Group 4, Losartan + ET-1. The second experiment includes: Group 1, Saline infusion, Group 2, Ang II infusion, Group 3, Losartan + Ang II, and Group 4, Bosentan + Ang II. The results from the experiment I demonstrated that bolus infusion of losartan significantly decreased serum cortisol, while bosentan slightly reduced it versus ET-1. Beside in experiment II, bosentan could significantly decrease cortisol compared with Ang II. Neither losartan nor Ang II changed serum cortisol significantly in comparison with Ang II and saline groups. Furthermore, bosentan caused rising in malondialdehyde (MDA) concentration compared to ET-1 infusion, but losartan slightly decreased it. MDA in Ang II infusion dramatically became high in comparison with saline infusion, and both losartan and bosentan non significantly returned it to the base line levels. Furthermore, serum glucose concentration clearly rose in losartan infusion, while bosentan did affect it significantly. Serum chloride in both bosentan and losartan significantly increased compared to ET-1. Both ET-1 and Ang II infusions for one hour led to increase Mg^{++} concentration in concomitant with saline infusion, while bosentan and losartan did not change it as compared with ET-1 and Ang II, respectively. **In conclusion**: Both ET-1 and Ang II antagonists reduced cortisol levels, but they did not change lipid peroxidation marker as elevated by Ang II infusion. Interestingly, ET-1 and Ang II markedly could increase serum Mg^{++} levels, but their antagonists did not return it to the normal levels.
[Sarkawt Hamad Ameen Hamad; Ismail Mustafa Maulood; Almas MR Mahmud. **Effect of Bosentan and Losartan on Oxidative Stress and Cortisol levels in Endothelin-1 and Angiotensin II Treated Rats.** *Life Sci J* 2016;13(10):43-49]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 6. doi:10.7537/marslsj131016.06.

Keywords: ET-1; Ang II; Cortisol; Magnesium

1. Introduction

Steroids among them glucocorticoids contribute in many physiological changes such as disease, trauma, and toxins which are well responded by corticoids (Fernandes et al. 2008). The cortisol hormone which secrets in adrenal cortex play important roles in developing oxidative stress and increases free radical level (Mercanoglu et al. 2008). While, there is little investigation about bosentan and losartan and their consequences on serum cortisol level and oxidative stress, but endothelin and angiotension II stimulate adrenocorticiod secretion (Rabano et al. 2004; Vierhapper et al. 1995)

On the other hand, ET-1 is widely synthesized in endothelial cell, it is well known as a potent vasoconstrictor by constricting blood vessels strongly (Lin et al. 2014). ET-1 has two receptor sub types, $ET\text{-}1_A$ which perform cell proliferation, vasoconstriction and many other physiological actions, thus through $ET\text{-}1_B$ would facilitate vasodilation (Maguire and Davenport 2014). For antagonizing ET-1 many synthetic antagonist have been produced to block single or dual ET-1 receptors, such as BQ123, BQ 788 and Bosentan, but only bosentan reach final step in pharmaceutical process, and used as a drug (Busnadiego et al. 2014). Bosentan is $ET\text{-}1_{A/B}$ dual receptor antagonist which is used to treat pulmonary hypertension (Markova et al. 2013).

Furthermore, Ang II secrets in many tissues, but generally it produces from a precursor of angiotensin I by action of angiotensin converting enzyme. It has a variety of physiological functions through binding with two different types of Ang II receptors (Barrett et al. 2010). Ang II subtype one receptor (AT_1) is responsible in enhancing cell proliferation, vasoconstriction, oxidative stress, production of other endocrine hormones, and increase Na^+ / K^+ pump activity (Lottermoser et al. 2003; Rabano et al. 2004; Seifi et al. 2014). While, stimulation of Ang II subtype two receptor (AT_2) lead to vasodilation, pro apoptotic, anti-inflammation and anti-growth (Savoia et al. 2011). Thus, one of the most anti hypertension drug is losartan, it could attenuate Ang II hypertensive

effects through antagonizing AT_1 receptor (Shiga et al. 2014).

Because of, the interactions between ET-1 and Ang II, and their antagonist actions on the body organs are the most controversial subjects among researchers, therefore the present work is to investigate, the effects of bosentan and losartan on oxidative stress and serum cortisol levels.

2. Materials and Methods

Animals

Albino rats (*Rattus norvegicus*) were bred in the animal house that belongs to Biology department, College of Science, Salahaddin University-Erbil. Forty-two male rats weighs between 300 – 400 grams have been used (six rats were kept for each plastic cage), overnight fasted (8-12) hr and animals were brought to the laboratory at the day of surgery. They were allowed to free access of tap water *ad libitum*

Anesthesia and control of the body temperature

The animals were anesthetized by intraperitonial injection of a mixture of Ketamine hydrochloride 80 mg / Kg (Trittau, Germany) and Xylazin 12 mg / Kg (Interchem, Holland). The depth of anesthesia was monitored by loosing reflexes. The anesthesia was remained for 1.5 – 2 hours and a supplement dose was used if necessary. The maximum volume anesthesia solution was 1ml / Kg; Rat's body temperature was controlled by placing on an electrical heating pad between 35 - 37 ^{o}C.

Procedure for tracheostomy

Tracheostomy was performed in order to achieve good ventilation and avoiding bronchial obstruction. Sterile forceps were used to pull up neck skin in the middle, and about 2 -3 cm incision was made longitudinally; layers of the neck skin were removed and cleaned from the connective tissue, the platysma muscle was dissected to observe trachea. Fine iris scissors were used to slit incision of the ventral part of trachea, it must be managed as fast as possible to prevent rat from bleeding and hypoxia, then polythene tube 2 mm OD, 4-5 cm was inserted and was tightened by ligature, for prevention of clot inside the tube it was wetted by heparinized normal saline 10 IU/ml. a syringe connected to a PE tube (ID 0.58 mm, OD 0.96 mm. England) had been used to control bronchial secretion, so any solutions present in the trachea removed by it.

Cannualation of femoral vein for intravenous infusion

Concave sterile scissors were used for making a small incision 2 - 3 cm on the right thigh, the outer layer of skin and matrix of collagen fibers had been removed, and cleaned carefully; a transient obstruction of blood back flow to the heart, it made the vein quite prominent, then a 27 G ½ needle filled with heparinized normal saline 10 IU / ml was inserted into the vessel which was 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 a 10 ml sized syringe, immediately after insertion of cannula normal saline was infused (15 ml / h /Kg). The administration of normal saline continued for hour, it called equilibration period, then the experimental design conducted; the wound was kept moisture by covered a gauze with heparinized normal saline.

Design of the experiments

The experimental design includes two sub experiments: Experiment A consisted of four groups, Group1, Saline (n = 6) animals were infused with normal saline (15 ml / h/ Kg) after equilibration period, Group 2, Endothelin-1 (n = 6) animals were infused with ET-1 (520 ng / min/ Kg), Group 3, Bosentan + Endothelin-1 (n = 6) the rats were infused bolus infusion of bosentan 10 mg/ 0.5ml / kg then continuously animals infused ET-1 (520 ng / min / Kg), and Group 4, Losartan + Endothelin-1 (n = 6) the rats were infused bolus infusion of losartan 10 mg/ 0.5 ml / kg then continuously animals infused ET-1 (520 ng / min/ Kg). Experiment B also consisted of four goups, Group 1, saline and treated as in experiment A, Group 2, Angiotensin II (n = 6) animals were infused Ang II (320 ng / min / Kg), Group 3, Losartan + Angiotensin II (n = 6) the rats were infused bolus infusion of losartan 10 mg/ 0.5 ml / kg then continuously animals infused Ang II (320 ng / min / Kg), and Group 4 Bosentan + Angiotensin II (n = 6) the rats were infused bolus infusion of bosentan 10 mg/ 0.5ml / kg then continuously animals infused with Ang II (320 ng / min / Kg).

Collection of blood samples

At the end of the sixty minutes of infusion about 7 ml of blood were obtained, through puncturing of heart, then standing in a clean clot activator gel test tube for 30 minutes; the blood centrifuged at 1000 g for 15 minute (Centromix-Mod. S-549), then serum was transferred into four clean eppendorf tubes and they were stored at -80 ^{o}C until assay (Sanyo – Ultra – Low Temperature, Japan).

Determination of serum cortisol

Enzyme immunosorbent assay(ELISA) method was used for determination of serum cortisol. The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for limited number of antibody sites.

Determination of serum MDA

An MDA-(Thiobarbituric acid)TBA was used to determine serum MDA, serum sample 150 µL and 1ml of 17.5% Trichloacetic acid (TCA) were added into clean centrifuge test tube (Supe-Rior, W-Germany), TCA was allowed to deproteinize specimen. One ml of 0.66% TBA added into same tube and it was mixed well by vortex (Vortex-Genie, Model K-550-Ge, USA). The sample was allowed for boiling at 95 ^{o}C in the water bath (Memmert, Gmbh+Co. KG, Germany) for a period of 45 minutes and left at room temperature 25 ^{o}C to cool, then 1 ml of 70% TCA added to precipitate the ruminant serum protein, centrifuged (Centromix-Mod. S-549) at 1000 g for 15 min, the pink colour indicate reaction was occurred, then read at 532 nm by spectrophotometer (Apel-PD303-Japan).

Determination of serum glucose

Oxidation of glucose was determined by enzymatic reactions using (Randox) kit. It produced a violet quinoneimine coluor absorbed by spectrophotometer (500 nm) which was proportioned to glucose concentration.

Determination of serum chloride

BioLabo (France) kit method was used to determine serum chloride by using spectrophotometer (Apel-PD303-Japab).

Determination of serum magnesium

Gindler, Heth and Khayam-Bashi method was used for determination of magnesium. The BioLabo (France) kit method was used to determine serum magnesium by using spectrophotometer (Apel-PD303-Japab).

2. Results

In the experiment A, bolus infusion of losartan significantly ($P < 0.05$) decreased serum cortisol level as compared with ET-1 group, while bosentan reduced cortisol level but it did not reach statistical consideration. There was no statistical difference between ET-1 and saline group (Figure 1, A). Beside that, in the experiment B, bosentan could significantly decrease cortisol level versus Ang II. Neither losartan nor Ang II changed serum cortisol significantly in comparison with Ang II and saline groups, respectively (Figure 1, B).

On the other hand, bosentan raised MDA concentration after ET-1 infusion ($P < 0.05$), but losartan slightly decreased it (Figure 2, A). MDA in Ang II infusion dramatically became high in comparison with saline infusion while, both losartan and bosentan non significantly returned it to the base line levels (Figure 2, B).

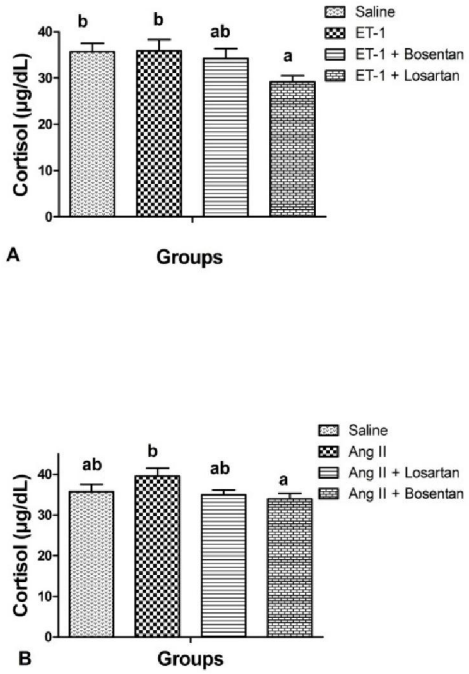

Figure 1: Serum cortisol in A, ET-1 treated rats. B, Ang II treated rats.

The different letters mean significant and the same letters mean no significant differences. The data mean ± SEM, $P < 0.05$ consider a significant difference according to 1-way ANOVA followed by Duncan post hoc test.

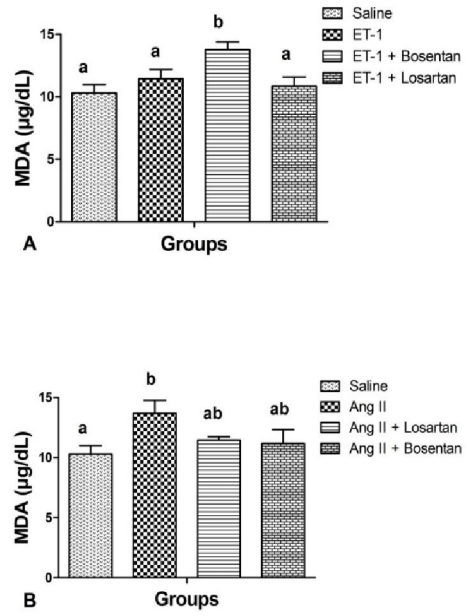

Figure 2: Serum MDA (µg/dL) in A, ET-1 treated rats. B, Ang II treated rats.

The different letters mean significant and the same letters mean no significant differences. The data mean ± SEM, P < 0.05 consider a significant difference according to 1-way ANOVA followed by Duncan post hoc test.

Table 1: Effect of ET-1 infusion on serum glucose, chloride and magenesium

Parameters / Group	Glucose (mg/dL)	Chloride * (mg/dL)	Magnesium * (mg/dL)
Saline	154.9 ± 15.00^{a}	97.41 ± 2.926^{a}	0.154 ± 0.0180^{a}
Ang II	161.2 ± 29.25^{a}	100.9 ± 0.777^{ab}	0.296 ± 0.025^{b}
Ang II + Losartan	143.8 ± 11.04^{a}	103.5 ± 3.120^{ab}	0.308 ± 0.040^{b}
Ang II + Bosentan	156.8 ± 20.64^{a}	106.0 ± 2.259^{b}	0.261 ± 0.044^{b}

Furthermore, serum glucose concentration clearly raised (P < 0.05) in losartan infusion and bosentan did that but not statistically (P > 0.05) (Table 1). While, serum chloride in both bosentan (111.2 ± 3.590) and losartan (114.1 ± 2.869) significantly increased it compared to ET-1 infusion (104.7 ± 2.990) (Table 1). Also, ET-1 infusion for one hour led to rise Mg^{+2} concentration in concomitant with saline infusion. (Table 1).

Table 2: Effect of Ang II infusion on serum glucose, chloride, magnesium

Parameters / Group	Glucose * (mg/dL)	Chloride * (mg/dL)	Magnesium* (mg/dL)
Saline	154.9 ± 15.00^{ab}	97.41 ± 2.926^{a}	0.154 ± 0.0180^{a}
ET-1	123.2 ± 8.087^{a}	104.7 ± 2.990^{ab}	0.242 ± 0.016^{b}
ET-1 + Bosentan	185.3 ± 25.35^{bc}	111.2 ± 3.590^{b}	0.264 ± 0.047^{b}
ET-1 + Losartan	212.9 ± 13.56^{c}	114.1 ± 2.869^{b}	0.227 ± 0.014^{ab}

The different letters mean significant and the same letters mean no significant differences. The data mean ± SEM * P < 0.05 considered a significant difference according to 1-way ANOVA followed by Duncan post hoc test.

Table 2 shows that, Mg^{++} was increased in bosentan (0.308 ± 0.040) and it was decreased in losartan (0.261 ± 0.044) as compared with Ang II (0.296 ± 0.025), but only bosentan could increases chloride (P < 0.05). There was no statistical change in glucose concentration in experimental B.

The different letters mean significant and the same letters mean no significant differences. The data mean ± SEM * P < 0.05 considered a significant difference according to 1-way ANOVA followed by Duncan post hoc test.

4. Discussion

The present study demonstrates that both ET-1 $_{A/B}$ dual (bosentan) and Ang II AT_1 (losartan) receptors attenuates cortisol concentration, have roles in oxidative stress and magnesium regulation, also bolus infusion of them affected chloride ion and glucose homeostasis. Losartan bolus infusion could reduce serum cortisol significantly as compared with ET-1infusion for one hour (Figure 1, A). Additionally, bosentan markedly decreased the elevated cortisol concentration caused by Ang II infusion (Figure 1, B). The exact mechanism by which losartan and bosentan caused cortisol reduction is not fully understood yet. However, our data agree with (Ansurudeen et al. 2014) which demonstrated that Ang II causes an increase in cortisol production. (Paramonova et al. 2010) reported that ET-1 causes proliferation of adrenocortical cells and promotes cortisol secretion. Furthermore, hyepercortisolemia has related with elevated ET-1 levels (Lederbogen et al. 1999). Our data disagree with (le Mevel et al. 1999) who demonstrated that intra-arterial injection of ET-1 did not markedly modify cortisol levels. Also, (Vierhapper et al. 1995) concluded that cortisol concentration was unchanged by infusion of ET-1. Experimental data for reducing cortisol levels by bolus infusion of the losartan are limited, while a study observed that Ang II modulated transcription regulatory genes of cortisol secretion and expression levels of unique enzymes of the glucocorticoid biosynthesis pathways (Rondon et al. 2014) In addition, Ang II directly stimulates adrenal cortisol production through releasing nitric oxide (Gauthier et al. 2005).

Although, ET-1 slightly elevated lipid peroxidation through serum MDA levels (Figure 2, A and B), while, Ang II increased it significantly as compared with the saline infusion. Interestingly, Bosentan infusion markedly increased MDA levels, whereas Losartan infusion did not change it significantly. It has been reported that the Ang II

infusion (Bild et al. 2013; Dianat et al. 2014) and ET-1 infusion would increase oxidative stress and alters the balance between oxidant (MDA level) and antioxidant enzymes (Fiore et al. 2005), and more recently (Lankhorst et al. 2014) demonstrated that activation of the ET-1 axis induces oxidative stress. However, at present there is no exact explanation for MDA elevation by bosentan administration, but a report indicated that, bosentan enhances hepatic toxicity and liver damage (Eriksson et al. 2011). Additionally, free radical production is strongly related with glucose metabolism, hence there is a report demonstrated that bosentan affects liver glycogen content and serum glucose (Said et al. 2005), however, our data is in contrast to (Demirci et al. 2015) who recently concluded that bosentan treatment improves diabetes – induces liver damage via oxidative stress reduction. Beside that, ET-1 infusion did not change serum glucose (Table 1), and the result may be due to ET-1 induces glucose uptake (Wu-Wong et al. 2002) and bosentan decreases serum glucose (Said et al. 2005), also, (Strawbridge and Elmendorf 2005) concluded that ET-1 induced insulin resistance and impaired glucose transport. While, Telmisartan can improve diabetic rats and insulin resistance (Younis et al. 2012), but losartan increases serum glucose.

Bolus infusion of both losartan and bosentan slightly increased serum chloride as compared with ET-1 infusion (Table 1). The possible mechanisms may be returned to inhibition of Na^+ - K^+ - $2Cl^-$ co-transporter and Cl^- / HCO_3^- exchanger (Dai and Zhang 2004). On the other hand, ET-1 inhibits thick ascending limb chloride flux via ET_B receptor mediated NO release (Plato et al. 2000), and ET-1 can potently stimulate chloride secretion (Kuhn et al. 1997).

Magnesium is an important physiological intracellular ion which has roles in cardiovascular regulation and it relaxes vessels and hence reduce blood pressure. (Finckenberg et al. 2005; Rondon et al. 2014). The present study (Table 1, and 2) showed that both ET-1 and Ang II infusion for one hours significantly increased serum magnesium. However, there is no previous report indicating such relation between magnesium ions and ET-1 and Ang II actions. However, many studies have been reported that magnesium ameliorate Ang II and ET-1 production (Berthon et al. 2003; Berthon et al. 2002; Ozturk et al. 2012). Although, Ang II infusion induced hypermagnesiuria, and hence reduces magnesium ions (Wu and Sonnenberg 1995), both ET-1 and Ang II increased magnesium, it is believed that this due to glomerular filtration rat (GFR) reduction and no more magnesium could be excreted through the urine but the exact explanation for this result needs further confirmation. **In conclusions,** both losartan and bosentan could reduce cortisol levels, and bosentan rather than losartan can elevate oxidative stress, also Ang II infusion could rise MDA levels more than ET-1 infusion. Interestingly, both ET-1 and Ang II can markedly elevate serum magnesium levels.

Acknowledgements:

We would like to thank Savano company – branch of Cipla – India company for their gift (Bosentan).

Corresponding Author:
Sarkawt Hamad Ameen Hamad
Department of Biology
Delzyan Campus, Soran University
Kurdistan region, Iraq
E-mail: sarkawt.hamad@soran.edu.iq

References

1. Ansurudeen, I., P. G. Kopf, K. M. Gauthier, S. R. Bornstein, A. W. Cowley, Jr. & W. B. Campbell, 2014. Aldosterone secretagogues increase adrenal blood flow in male rats. Endocrinology 155(1):127-32.
2. Barrett, K. E., S. M. Barman, S. Boitano & H. L. Brooks, 2010. Ganong's review of medical physiology McGraw-Hill, United States of America Twenty third edition.
3. Berthon, N., P. Laurant, D. Fellmann & A. Berthelot, 2003. Effect of magnesium on mRNA expression and production of endothelin-1 in DOCA-salt hypertensive rats. J Cardiovasc Pharmacol 42(1):24-31.
4. Berthon, N., P. Laurant, D. Hayoz, D. Fellmann, H. R. Brunner & A. Berthelot, 2002. Magnesium supplementation and deoxycorticosterone acetate--salt hypertension: effect on arterial mechanical properties and on activity of endothelin-1. Can J Physiol Pharmacol 80(6):553-61.
5. Bild, W., L. Hritcu, C. Stefanescu & A. Ciobica, 2013. Inhibition of central angiotensin II enhances memory function and reduces oxidative stress status in rat hippocampus. Prog Neuropsychopharmacol Biol Psychiatry 43:79-88.
6. Busnadiego, O., J. Loureiro-Alvarez, P. Sandoval, D. Lagares, J. Dotor, M. L. Perez-Lozano, M. J. Lopez-Armada, S. Lamas, M. Lopez-Cabrera & F. Rodriguez-Pascual, 2014. A Pathogenetic Role for Endothelin-1 in Peritoneal Dialysis-Associated Fibrosis. J Am Soc Nephrol 10:2013070799.

7. Dai, Y. & J. Zhang, 2004. Chloride efflux is involved in ET-1 and 5-HT-induced contraction in rabbit basilar artery. J Cardiovasc Pharmacol 44(1): S125-8.
8. Demirci, E., I. Ferah, C. Gundogdu, S. Ozkanlar, N. K. Baygutalp, Y. Bayir, M. Calik & G. Ayaz, 2015. Endothelin receptor inhibition with bosentan delays onset of liver injury in streptozotocin-induced diabetic condition. Drug Res 65(5):272-80.
9. Dianat, M., G. R. Hamzavi, M. Badavi & A. Samarbafzadeh, 2014. Effects of losartan and vanillic Acid co-administration on ischemia-reperfusion-induced oxidative stress in isolated rat heart. Iran Red Crescent Med J 16(7):5.
10. Eriksson, C., A. Gustavsson, T. Kronvall & C. Tysk, 2011. Hepatotoxicity by bosentan in a patient with portopulmonary hypertension: a case-report and review of the literature. J Gastrointestin Liver Dis 20(1):77-80.
11. Fernandes, D., D. Duma & J. Assreuy, 2008. Steroids and nitric oxide in sepsis. Front Biosci 13:1698-710.
12. Finckenberg, P., S. Merasto, M. Louhelainen, L. Lindgren, H. Vapaatalo, D. N. Muller, F. C. Luft & E. M. Mervaala, 2005. Magnesium supplementation prevents angiotensin II-induced myocardial damage and CTGF overexpression. J Hypertens 23(2):375-80.
13. Fiore, G., P. Florio, L. Micheli, C. Nencini, M. Rossi, D. Cerretani, G. Ambrosini, G. Giorgi & F. Petraglia, 2005. Endothelin-1 triggers placental oxidative stress pathways: putative role in preeclampsia. J Clin Endocrinol Metab 90(7):4205-10.
14. Gauthier, K. M., D. X. Zhang, E. M. Edwards, B. Holmes & W. B. Campbell, 2005. Angiotensin II dilates bovine adrenal cortical arterioles: role of endothelial nitric oxide. Endocrinology 146(8):3319-24.
15. Kuhn, M., M. Fuchs, F. X. Beck, S. Martin, J. Jahne, J. Klempnauer, V. Kaever, G. Rechkemmer & W. G. Forssmann, 1997. Endothelin-1 potently stimulates chloride secretion and inhibits Na(+)-glucose absorption in human intestine in vitro. J Physiol 499(Pt 2):391-402.
16. Lankhorst, S., M. H. Kappers, J. H. van Esch, A. H. Danser & A. H. van den Meiracker, 2014. Hypertension during vascular endothelial growth factor inhibition: focus on nitric oxide, endothelin-1, and oxidative stress. Antioxid Redox Signal 20(1):135-45.
17. le Mevel, J. C., C. Delarue, D. Mabin & H. Vaudry, 1999. Central and peripheral administration of endothelin-1 induces an increase in blood pressure in conscious trout. Am J Physiol 276(4 Pt 2): R1010-7.
18. Lederbogen, F., B. Weber, M. Colla, I. Heuser & M. Deuschle, 1999. Endothelin-1 plasma concentrations in depressed patients and healthy controls. Neuropsychobiology 40(3):121-3.
19. Lin, Y. J., C. F. Kwok, C. C. Juan, Y. P. Hsu, K. C. Shih, C. C. Chen & L. T. Ho, 2014. Angiotensin II enhances endothelin-1-induced vasoconstriction through upregulating endothelin type A receptor. Biochem Biophys Res Commun 451(2):263-9.
20. Lottermoser, K., T. Unger, P. Gohlke, H. Vetter & R. Dusing, 2003. Differential effect of acute angiotensin II type 1 receptor blockade on the vascular and adrenal response to exogenous angiotensin II in humans. Am J Hypertens 16(6):445-52.
21. Maguire, J. J. & A. P. Davenport, 2014. Endothelin@25 - new agonists, antagonists, inhibitors and emerging research frontiers: IUPHAR Review 12. Br J Pharmacol 18(10):12874.
22. Markova, S. M., T. De Marco, N. Bendjilali, E. A. Kobashigawa, J. Mefford, J. Sodhi, H. Le, C. Zhang, J. Halladay, A. E. Rettie, C. Khojasteh, D. McGlothlin, A. H. Wu, W. C. Hsueh, J. S. Witte, J. B. Schwartz & D. L. Kroetz, 2013. Association of CYP2C9*2 with bosentan-induced liver injury. Clin Pharmacol Ther 94(6):678-86.
23. Mercanoglu, G., N. Safran, H. Uzun & L. Eroglu, 2008. Chronic emotional stress exposure increases infarct size in rats: the role of oxidative and nitrosative damage in response to sympathetic hyperactivity. Methods Find Exp Clin Pharmacol 30(10):745-52.
24. Ozturk, C. F., C. Karakelleoglu, Z. Orbak & L. Yildiz, 2012. The effect of serum magnesium levels and serum endothelin-1 levels on bone mineral density in protein energy malnutrition. West Indian Med J 61(3):213-8.
25. Paramonova, I., M. Haase, B. Mulders-Opgenoorth, I. Ansurudeen-Rafi, S. R. Bornstein, C. Papewalis, S. Schinner, M. Schott, W. A. Scherbaum & H. S. Willenberg, 2010. The effects of the endothelium on adrenal steroidogenesis and growth are mainly mediated by proteins other than endothelin-1. Horm Metab Res 42(12):840-5.
26. Plato, C. F., D. M. Pollock & J. L. Garvin, 2000. Endothelin inhibits thick ascending limb chloride flux via ET(B) receptor-mediated NO release. Am J Physiol Renal Physiol 279(2): F326-33.
27. Rabano, M., A. Pena, L. Brizuela, J. M. Macarulla, A. Gomez-Munoz & M. Trueba,

2004. Angiotensin II-stimulated cortisol secretion is mediated by phospholipase D. Mol Cell Endocrinol 222(1-2):9-20.
28. Rondon, L. J., E. Marcano, F. Rodriguez & J. R. del Castillo, 2014. Blood pressure, magnesium and other mineral balance in two rat models of salt-sensitive, induced hypertension: effects of a non-peptide angiotensin II receptor type 1 antagonist. Magnes Res 27(3):113-30.
29. Said, S. A., S. M. Ammar el & G. M. Suddek, 2005. Effect of bosentan (ETA/ETB receptor antagonist) on metabolic changes during stress and diabetes. Pharmacol Res 51(2):107-15.
30. Savoia, C., M. D'Agostino, F. Lauri & M. Volpe, 2011. Angiotensin type 2 receptor in hypertensive cardiovascular disease. Curr Opin Nephrol Hypertens 20(2):125-32.
31. Seifi, B., M. Kadkhodaee, E. Bakhshi, M. Ranjbaran, M. Zahmatkesh, Z. Sedaghat, P. Ahghari & P. Esmaeili, 2014. Angiotensin II in paraventricular nucleus contributes to sympathoexcitation in renal ischemia-reperfusion injury by AT1 receptor and oxidative stress. J Surg Res 5(14):00620-9.
32. Shiga, Y., S. I. Miura, K. Norimatsu, Y. Hitaka, I. Nagata, R. Koyoshi, J. Morii, T. Kuwano, Y. Uehara, A. Inoue, T. Shirotani, K. Fujisawa, E. Matsunaga & K. Saku, 2014. Efficacy and safety of combination therapy of high-dose losartan and hydrochlorothiazide in patients with hypertension. J Renin Angiotensin Aldosterone Syst 20:1470320314529358.
33. Strawbridge, A. B. & J. S. Elmendorf, 2005. Phosphatidylinositol 4,5-bisphosphate reverses endothelin-1-induced insulin resistance via an actin-dependent mechanism. Diabetes 54(6):1698-705.
34. Vierhapper, H., P. Nowotny & W. Waldhausl, 1995. Effect of endothelin-1 in man: impact on basal and adrenocorticotropin-stimulated concentrations of aldosterone. J Clin Endocrinol Metab 80(3):948-51.
35. Wu-Wong, J. R., C. E. Berg & B. D. Dayton, 2002. Endothelin-stimulated glucose uptake: effects of intracellular Ca(2+), cAMP and glucosamine. Clin Sci 103(48):418S-423S.
36. Wu, X. & H. Sonnenberg, 1995. Effect of renal perfusion pressure on excretion of calcium, magnesium, and phosphate in the rat. Clin Exp Hypertens 17(8):1269-85.
37. Younis, F., Y. Oron, R. Limor, N. Stern & T. Rosenthal, 2012. Prophylactic treatment with telmisartan induces tissue-specific gene modulation favoring normal glucose homeostasis in Cohen-Rosenthal diabetic hypertensive rats. Metabolism 61(2):164-74.

10/18/2016