Effects of bosentan and losartan on 2k1c hypertensive rats

Effects of bosentan and losartan on kidney and liver weight in 2K1C hypertensive rats.

Introduction

Hypertension (HTN) has important contribution in cardiovascular risk factors, it has great consideration by the researchers, therefore, several animal models have been developed to investigate consequences of HTN. The most well known model is Goldblatt model. Which is a widely used for renovascular HTN (Badyal *et al* ., 2003). The 2K1C Goldblatt model is induced by using silver clip on unilateral stenosis of the renal artery and the other kidney untouched (Chabielska *et al* ., 2000).

The 2k1C Goldblatt model decrease the renal function (Sporkova *et al.,* 2011) through elevation plasma Ang II, several potential interactions are revealed between ET-1 and Ang II, ET-1 can stimulate aldosterone secretion and increasing adrenal cortex cell proliferation (Rossi *et al.* , 2000).

In addition, ET-1 in the hypertension is controversial subject among researchers over two decades from ET-1discovery time (Yanagisawa *et al.,* 1988 *;* Dhaun *et al* ., 2008). Schiffrin (1995) Reported that plasma ET-1 concentration is elevated in essential hypertention and Goldblatt model. It is responsible for constricting vascular beds, increase smooth muscle hypertrophy and proliferation and leads to increase vascular resistance, decline renal function and then hypertension. Giersbergen (2002) said that ET-1 and Ang II, they are increases catabolic enzymes which are responsible on growth regulation. When, Ang II antagonist given rats caused decreasing drinking behavior such as losartan because, it could pass blood – brain –

barrier reduce response to intraventricular angiotensin II (Palmer *et al* ., 1994)

The identification of ET receptor subtypes led to understand the types and their structure. They participated greatly in pathophysiology, for that reason scientists attempt to block ET signal receptors by peptide and non peptide substances. Davenport and Battistini (2002) revealed that ET receptor antagonists are currently classified as ET_A selective (BQ 123), ET_B selective (BQ 788), or mixed antagonists that display similar affinities for both receptor subtypes. However, only ET_A selective or ET_A / ET_B (Bosentan) antagonists are currently being evaluated in clinical trials.

There are little known about the physiological actions of Endothelin-1 and Angiotensin II on kidney and liver weight in 2K1C induced hypertensive rats and there are few knowledge about their antagonists (bosentan and losartan) on 2K1C rat models. For that reason the following object was performed in the present study.

Materials and Methods.

Animals

Albino rats (*Rattus norvegicus*) were bred in the animal house that belongs to Biology Dept. /College of Science/University of Salahaddin/ Hawler. After many generation of breeding, experiments were performed on ninety nine male rats weights between 200 – 400 grams. Male rats were used in preference female rats, because female resistance for hypertension due to high amount of estrogen (Cotter *et al* ., 1990). Animals were given standard

rat diet. The room temperature controlled in range 22±2 C ^{o} and 12/12 dark cycle photoperiod.

Experimental design

This experiment was designed to develop renovascular hypertensive rats throught two kidney one clip (2K1C) Goldblatt model, then treating them by bosentan which is ET-1 $_{A/B}$ receptors antagonist and Losartan (Ang II receptor A antagonist). The goal of this experiment was performed by five groups of rats. Each of them has same animal house conditions. They were different according to follow situations

Group 1: Sham rats (n= 9)

Rats underwent left abdominal incision and all surgical procedure without using silver clip. They were given one ml normal saline (0. 9%) by gavage every day between 8 am to 9: 30 am for four weeks.

Group 2: Normotensive (2K1C $_{(n)}$) rats (n= 7)

Rats had silver clip around left renal artery would not developed hypertension. They were administered normal saline (0. 9%)by gavage every day between 8 am to 9: 30 am for four weeks.

Group 3: 2K1C $_{(h)}$ rats (n= 7)

Rats had silver clip around left renal artery with developed hypertension. They were taken normal saline (0. 9%) every day between 8 am to 9: 30 am for four weeks.

Group 4: 2K1C $_{(h)}$ + Bosentanrats (n= 8)

Rats had silver clip around left renal artery developed hypertension then treated by bosentan 30mg/kg (Cipla, India) every day between 8 am to 9: 30 am by gavage after three weeks of surgery from developed hypertension continued for four weeks (Lee *et al* ., 2007)

Group 5: 2K1C $_{(h)}$ + Losartan(n= 8)

Rats had silver clip around left renal artery developed hypertension then Losartan (Actavis, Icelanda) was administrated by gavaging 30mg/kg every day between 8 am to 9: 30 am after three weeks of surgery from developed hypertension continued until four weeks (Moosavi and Johns, 1999).

Preparation of animals for 2K1C Goldblatt hypertension

Animals were anesthetized by injection with mixture of Ketamine hydrochloride 80mg/Kg (Trittau, Germany) and Xylazin 12mg/Kg (Interchem, Holland) intraperitonealy (Reineke *et al* ., 2003). Left abdominal side was cleaned from fur, sterilized by idophore and 4-5cm flank incision was made to expose left kidney then carefully dissected and freed left renal artery from left renal vein. AUshaped silver clip internal diameter (0. 25-0. 3) mm was placed around left renal artery, causing partial occlusion, decrease 70% blood flow. The right kidney was untouched (Sigmon, and Beierwaltes, 1993).

After, the clipping process was successfully performed, the left kidney was slowly pushed back into retroperitoneal cavity, the wound was cleaned by sterilized solution, the abdominal muscle was sutured by 3. 0 gage chromic gut suture and the abdominal skin sutured by Ethilon monofilament nylon

suture 3. 0 gage. The wound again was sterilized then gauze placed above the sewed wound. Continuous heating was done to animals for 24 hours and they were kept inside a cage with free access for food and water. The whole procedure on sham group were done except clipped silver clip around left renal artery (Cotter *et al* ., 1990).

Organ weights

Abdominal skin and muscle of rats were removed by curved scissors. Rat's organs were separated, cut and transferred into a clean petridish. Weighed (Mettler PM200) and recorded. Then, rat's organ weight mathematically converted to per 100 body weight.

Collection of Urine samples

After three weeks of surgery, the animals were weighed and kept in the urine collector individually with water (100 ml) and standard rodent diet 20 grams. In the next day, the amount of urine was collected and stored in refrigerator until assay. It was repeated once a week for four weeks.

Water and food intake

Animals were left in urine collector and ambient conditions were controlled, after 24 hours amount of water intake measured (100 ml – amount of water drunk) and amount of food intake measured (20 g – amount of food eaten). This was continued for four weeks.

Results

As shown (Table 4. 8) administration of bosentan and losartan caused a significant reduction in body weight at week six after surgery (248. 3 ± 21.

74, 256. 7 ± 10. 86), respectively as compared with 2K1C $_{(h)}$ (304. 7 ± 8. 554).

The process of clipping for eight weeks on left renal artery caused a significant decrease in left kidney weight compared with sham group. However, such reduction of weight in 2K1C $_{(h)}$ rats (0. 0025 ± 0. 0004) was more than 2K1C $_{(n)}$ rats (0. 0080 ± 0. 001) Table (4. 9). Also, constriction left renal artery caused increases (P <0. 05) in right kidney weight in 2K1C $_{(h)}$ rats as compared with sham. While, losartan and bosentan could decrease right kidney significantly (P <0. 05) in comparison with 2K1C $_{(h)}$ rats. In addition, both bosentan (0. 0553 ± 0. 0035) and losartan (0. 0578 ± 0. 0078) administration would decrease in liver weight which was statistically significant (P <0. 05) in comparison with hypertensive rats (0. 0756 ± 0. 0027). While there were no significant changes among nomotensive, hypertensive and sham group (Table 4. 9).

	Three week s*	Four week s**	Five weeks *	Six weeks *
Groups	after surgery	after surgery	after surgery	after surgery
Sham		283. 7 ± 8.	307. 4 ± 33.	309. 6 ± 13.

	523 [b]	453 [b]	15 [b]	19 [b]
2K1C [(n)]	221. 5 ± 18. 08 [a]	231. 0 ± 8. 047 [a]	286. 2 ± 34. 96 [ab]	248. 4 ± 10. 87 [a]
2K1C [(h)]	252. 14 ± 9. 103 [ab]	256. 0 ± 13. 33 [ab]	299. 1 ± 12. 42 [ab]	304. 7 ± 8. 554 [b]
2K1C [(h)] + Bosentan	265. 1 ± 15. 66 [b]	254. 8 ± 16. 80 [ab]	262. 6 ± 16. 26 [a]	248. 3 ± 21. 74 [a]
2K1C [(h)] + Losartan	274. 3 ± 10. 39 [b]	256. 3 ± 12. 39 [ab]	270. 0 ± 7. 000 [ab]	256. 7 ± 10. 86 [a]

Table 4. 9: Effects of bosentan and Losartan on weights of left kidney, right kidney and liver from third week to sixth week after surgery in 2K1C hypertensive rats.

Weeks Groups	Left Kidney * Per 100g	Right Kidney * Per 100g)	Liver* Per 100g
Sham	0. 0105 ± 0. 0005 [c]	0. 0106 ± 0. 0004 [a]	0. 0811 ± 0. 0037 [b]
2K1C (n)	0. 0080 ± 0. 0017 [b]	0. 0120 ± 0. 0012 [a]	0. 0768 ± 0. 0050 [b]
2K1C (h)	0. 0025 ± 0. 0004 [a]	0. 0148 ± 0. 0008 [b]	0. 0756 ± 0. 0027 [b]
2K1C (h) + Bosentan	0. 0021 ± 0. 0001 [a]	0. 0115 ± 0. 0007 [a]	0. 0553 ± 0. 0035 [a]
2K1C (h) +	0. 0020	0. 0124	0.

Losartan	± 0. 0003 [a]	± 0. 0008 [a]	0578 ± 0. 0078 [a]

Water intake in losartan administration rats significantly (P <0. 05) decreased (10. 36 ± 2. 553) in week three but in other weeks not significantly changed. Also, bosentan administration caused a significant decrease in water intake in weeks four and six (7. 377 ± 0. 8090 and 5. 473 ± 1. 066) respectively. The water intake in normotensive rats changed but not significantly. On the other hand, water intake in the 2K1C normotensive rats significantly increased in week four after surgery compared with sham (Table 4. 10).

Food intake was measured every weeks, it has been started from week three post surgery. Table 4. 11 shows that in 2K1C $_{(n)}$ group there was a significant increase in food intake at week three but there were no significant differences in weeks four, five and six. Food intake at week four after surgery tended to decrease significantly in 2K1C $_{(h)}$ rats treated with losartan and bosentan administration.

Collection of urine flow performed once weekly from week three to week six after surgery, the results are shown in the table 4. 12. It was illustrated that there was slight reduction of urine flow in 2K1C as compared with sham group especially in five and six weeks after surgery. Also, bosentan caused a slight non significant decrease in urine flow in the last two urine sample collections.

Table 4. 10: Effects of bosentan and losartan on water intake (ml/100g b. w) from third week to sixth week after surgery in 2K1C hypertensive rats.

Weeks Groups	Three weeks* after surgery	Four weeks* after surgery	Five weeks* after surgery	Six Week* after surgery
Sham	15. 06 ±3. 305 ab	9. 327 ±2. 778 ab	14. 11 ±0. 9777 b	8. 198 ±0. 738 ab
2K1C (n)	16. 37 ±2. 325 ab	14. 46 ±1. 151 c	11. 82 ±1. 044 ab	11. 17 ±2. 057 b
2K1C (h)	20. 62 ±3.	12. 51 ±1. 093	10. 21 ±1. 324	13. 59 ±1. 541 b

	177 b	bc	ab	
2K1C (h) + Bosentan	12. 87 ±0. 803 ab	7. 377 ±0. 8090 a	6. 925 ±0. 8343 a	5. 473 ±1. 066 a
2K1C (h) + Losartan	10. 36 ±2. 553 a	13. 58 ±2. 664 bc	11. 87 ±3. 592 ab	12. 44 ±3. 033 b

Table 4. 11: Effects of bosentan and losartan on food intake (g/100g b. w) from third week to sixth week after surgery in 2K1C hypertensive rats.

Weeks Groups	Three weeks* after surgery	Four weeks* after surgery	Five weeks after surgery	Week sixs* after surgery
Sham	3. 575 ±0.	5. 449 ±0.	3. 708 ± 0.	5. 177 ±0.

	2535			
	288 b	399 a	397 b	
a				
	5.	4.	4.	
	350 ±	921	832 ±	4. 735
2K1C (n)	0.	±0.	0.	±0.
	778 b	625 b	234 a	563 b
	4.			
	683 ±	4.	10.	4. 058
	932 ±	31 ±		
2K1C (h)	0.	0.	0.	± 0.
	458			474 ab
ab	642 b	626 a		
	5.			
	451 ±	3.	5.	2. 888
2K1C (h)	330 ±	178 ±		
+	1.	0.	0.	± 0.
Bosentan	2790			677 a
b	609 a	616 a		
	4.			
	842 ±	3.	4.	4. 295
2K1C (h)	134 ±	524 ±		
+	1.	0.	0.	± 0.
Losartan	456			465 ab
ab	403 a	520 a		

Table 4. 12: Effects of bosentan and losartan on urine flow (ml/Kg/hr) from third week to sixth week after surgery in 2K1C hypertensive rats.

Weeks / Groups	Three weeks after surgery	Four weeks after surgery	Five weeks* after surgery	Six weeks after surgery
Sham	0.9195 ±0.2760a	0.7317 ±0.1381a	1.280 ±0 .2036b	1. 482 ±0.5072a
2K1C (n)	0.7280 ±0.2598a	0.7052 ±0.3050a	0.7286 ±0.1786ab	0.6325 ±0.2346a
2K1C (h)	1.373 ±0.3616	0.7718 ±0.1376	1.033 ±0.2383	0.9083 ±0.1582

	a	a	ab	a
	0.	1.	0.	0.
2K1C (h)	8382	162	5971	6880
+	±0.	±0.	±0.	±0.
Bosentan	1866	2976	0938	1200
	a	a	a	a
	1.	0.	0.	0.
2K1C (h)	022	6813	8900	8550
+	±0.	±0.	±0.	±0.
Losartan	2027	1564	1278	1189
	a	a	ab	a

Discussion

The present data showed that administration of bosentan and losartan could decrease body weight significantly (P <0. 05). The possible mechanism of both antagonists to reduce body weight, might be due to interfering RAS (Al-Thanoon, and Mahmood, 2012), because they act to decrease positive feedback between ET-1 and Ang II, which they are increases catabolic enzymes which are responsible on growth regulation (Giersbergen *et al.* , 2002). Also, this study showed that (Table 4. 8) weight was significantly (P <0. 05) decreased in 2K1C normotensive compared with sham group. It is for the first time we confirm the idea, which was believed that RAS is responsible on weight gain, as in normotensive rats have slow responsiveness to Ang II effects (Melaragno, andFink, 1996).

It is shown in the current study that reduction in blood flow through clipping renal artery could decrease the weight of left kidney in hypertensive rats. Neither bosentan nor losaratn administration restored the weight (Table 4. 9). One of the possible mechanisms of decreasing left kidney weight may be due to the reduction in supply of oxygen to the kidney cells and waste substances accumulation that lead to reduction in the tissue growth (Nathan and Singer, 1999). While, in normotensive rats left kidney weight significantly (P <0. 05) greater than hypertensive rats. It means that another factor contributed in decreasing left kidney weight, but still not clear obvious (Table. 4. 9).

On the other hand, left renal artery constriction caused significant (P <0. 05) increase in right kidney weight. It means that RAS, which was activated by clipping process of the left kidney has hypertrophic effects on right kidney, this data was in parallel with study of (Sporková *et al* ., 2011). Also, for the first time we showed that both Ang II and ET-1 are responsible for right kidney hypertrophy, because both bosentan and losartan administration orally every day could significantly (P <0. 05) reduce right kidneys weight towards normal.

Furthermore, daily administration of bosentan markedly decreased liver weight. The reason behind that, is bosentan might cause liver injury as proven in our result, which were increased AST and decreased ALP activities in serum Gabbay, *et al* . (2007). The exact reason of reduced liver weight is not well established yet, but according to our knowledge the mechanism may also be due to remove Ang II effects on liver cells through blocking AT $_1$

Water intake once a week was measured, when temperature and light cycle (12/12 h) controlled. The results showed that in week three losartan treatment caused a marked reduction in water intake. One hypothesis mechanism to interpret that, is losartan could pass blood-brain-barrier, block AT_1 and then reduce in drinking response to intraventricular angiotensin II (Palmer *et al* ., 1994). Also, bosentan at weeks six caused significant decreases in water intake. But why losartan effects appeared at three weeks after surgery and bosentan effects observed at sixth week after surgery is not well established. However, one possible mechanism of this new finding is that their pharmacokinetics are different (Giersbergen *et al* ., 2002). Ang II and ET-1 receptor down regulations also might be involved in this mechanism, but also it needs further pharmacological studies to confirm it.

Although, reducing effects of bosentan and losartan on 2K1C hypertensive rats in food intake, were observed in the current results. The possible mechanism behind that is both of them may decrease the consequences of Ang II and ET-1 on hormones, which are related to glucose regulation and metabolism. In addition, food intake increased in normotensive rats, may be due to increasing hydrolytic enzymes, which required energy, for that it need organic molecules to produce energy, as performed in the food. This clearification is supporting our results as ALP is increased (Table 4. 3). Rat urine was collected for twenty four hours and then urine flow per minute was drawn out from that amount of urine. After comparison between groups, urine flow was slightly decreased in $2K1C_{(n)}$ group, due to reducing effects of Ang II on pressure diuresis. Also, bosentan caused none significant

reduction in urine flow. Possibly, this effect may be as the result of decreasing in water intake.