報告番号	香大医博甲　第 713号

様式１０７

学位論文審査の結果の要旨

平成　31　年　1　月　　21日

審査委員	主　査	中村 隆範		印
	副主査	村尾 孝児		印
	副主査	白神 豪太郎		印
願出者	専攻	分子情報制御医学専攻	部門	生体情報学
	学籍番号	15D745	氏名	李　磊
論文題目	Effects of the novel nonsteroidal mineralocorticoid receptor blocker, esaxerenone (CS-3150), on blood pressure and urinary angiotensinogen in low-renin Dahl salt-sensitive hypertensive rats.			
学位論文の審査結果	(合格)　・　不合格　　(該当するものを○で囲むこと。)			

〔 要　旨 〕

Herein, we studied the effects of the novel nonsteroidal selective mineralocorticoid receptor (MR) blocker, esaxerenone, on blood pressure and renal injury in Dahl salt-sensitive (DSS) rats. We also monitored intact urinary and total angiotensinogen (AGT). DSS rats were given a normal salt diet (NS: 0.5% NaCl, n = 10), a high-salt diet (HS: 8% NaCl, n = 10), HS + esaxerenone (1 mg/kg/day, p.o., n = 10), or HS + losartan (angiotensin II receptor blocker, 10 mg/kg/day, p.o., n = 10) for 6 weeks. Glomerular and tubulointerstitial tissues were obtained via a laser capture method. HS-treated DSS rats developed hypertension, albuminuria, and glomerular injury, which were associated with increased glomerular desmin staining and reduced mRNA levels of glomerular podocin and nephrin. HS-treated DSS rats also showed tubulointerstitial fibrosis with an increase in renal oxidative stress (4-hydroxynonenal staining). The urinary ((total AGT−intact AGT)/intact AGT) ratio, an indicator of intrarenal renin activity, was significantly suppressed in HS-treated DSS rats. Treatment with esaxerenone significantly decreased blood pressure, while losartan did not. Furthermore, esaxerenone attenuated the development of albuminuria, glomerular injury, and tubulointerstitial fibrosis more than losartan did, and this effect was associated with reduced renal oxidative stress. These data indicate that esaxerenone has antihypertensive and renal protective effects in salt-dependent hypertensive rats with suppressed intrarenal renin activity, as indicated by low levels of the urinary (total AGT−intact AGT)/intact AGT ratio.

Nonsteroidal mineralocorticoid receptor blockers (MRBs) have been recently developed, which have high selectivity and affinity to block MR as compare to steroidal MR antagonists, such as spironolactone and eplerenone. The present study examined the effects of the nonsteroidal MRB, esaxerenone, and angiotensin receptor blocker (ARB), losartan, on blood pressure and renal injury in DSS hypertensive rats. Several concerns have been raised regarding our studies, as follows,

1. Doses of esaxerenone and losartan:

It has been reported that esaxerenone (1 mg/kg/day) sufficiently decreases blood pressure in DSS rats. Furthermore, previous studies have shown that losartan (10 mg/kg/day) significantly decreased blood pressure in spontaneously hypertensive rats. Based on these data, we used esaxerenone and losartan at 1 and 10 mg/kg/day, respectively, in DSS rats.

2. Experimental protocols and methods for blood sampling:

In our study, we used five-week-old male DSS rats. DSS rats were treated with a normal salt (NS) diet (NS: 0.5% NaCl) or an HS diet (8% NaCl). HS- and NS-fed DSS rats were divided into three groups as follows: (1) vehicle, (2) esaxerenone and (3) losartan. After 6 weeks their treatments, animals were anesthetized by isoflurane and blood was quickly collected in EDTA-containing tubes by using a catheter via the abdominal aorta. Whole blood was centrifuged at 4°C for 10 min to separate the plasma. Plasma samples were stored at −30 °C.

3. Renin-angiotensin system (RAS) and biomarker

HS diet decreases plasma renin activity (PRA) and aldosterone levels. However, recent studies have shown that MR is directly activated by salt, independently of RAS. Although plasma potassium level is also an important modulator of plasma aldosterone, we did not measure it in this study. Diagnosis of low-renin salt-sensitive hypertensive subjects by measuring PRA is not easy because of its instability. Furthermore, it is difficult to monitor renin activity in the kidney. In this regard, we have established a method to calculate renin activity by urinary ratio of des(angiotensin I)AGT (calculated by total AGT minus intact AGT) to intact AGT, a potential stable indicator of intrarenal renin activity. Our data indicate that urinary ratio of (total AGT - intact AGT)/ intact AGT is a useful biomarker to identify low-renin salt-sensitive hypertensive patients who show a resistance to an ARB.

4. Mechanisms responsible for MRB-dependent renal protection:

In this study, MRB significantly decreased blood pressure and renal injury in DSS hypertensive rats. Therefore, MRB-dependent renal protective effects will be elicited, at least in part, by blood pressure reduction. However, our data showed that MRB significantly decreased renal oxidative stress which was associated with reductions in NADPH-oxidase subunits, p22phox and gp47phox gene expression in renal tissues. Thus, it is also possible that the antioxidative effects of MRB contribute to its renal protection.

5. **Conclusions:**

In conclusion, the present study demonstrates that the novel nonsteroidal MRB, esaxerenone, elicits antihypertensive and renal protective effects in rats with low-renin salt-dependent hypertension, which is associated with its antioxidative effect. These data support the hypothesis that nonsteroidal MR blockade is a novel therapeutic strategy for salt-sensitive hypertension and associated renal disease.

掲 載 誌 名	Hypertension Research　　第　　巻, 第　　号		
(公表予定) 掲 載 年 月	2018年12月	出版社（等）名	Nature

(備考) 要旨は，１，５００字以内にまとめてください。