Kobe University Repository : Kernel

PDF issue: 2026-11-03

Heme-oxygenase upregulation ameliorates angiotensin II induced tubulointerstitial injury and salt-sensitive hypertension

PRADHAN, ASHOK

(Degree)
博士（医学）

(Date of Degree)
2007-03-25

(Resource Type)
doctoral thesis

(Report Number)
甲3990

(URL)
https://hdl.handle.net/20.500.14094/D1003990

※ 当コンテンツは神戸大学の学術成果です。無断複製・不正使用等を禁じます。著作権法で認められている範囲内で、適切にご利用ください。

【 128 】

氏　　名・（本　籍）	ASHOK　PRADHAN　（　ネパール　）
博士の専攻分野の名称	博士（医学）
学　位　記　番　号	博い第1826号
学位授与の　要　件	学位規則第５条第１項該当
学位授与の　日　付	平成19年3月25日

【 学位論文題目 】

Heme-oxygenase upregulation ameliorates angiotensin Ⅱ induced tubulointerstitial injury and salt-sensitive hypertension
（ヘムオキシゲナーゼ発現増強は、アンギオテンシンⅡによる尿細管間質病変と食塩感受性高血圧を軽減する）

審　査　委　員

主 査　教　授　横山　光宏
教　授　横野　浩一
教　授　秋田　穂束

Summary

Hypertension is a multi-factorial disease and the leading risk factor for various cardiovascular and renal complications such as stroke, myocardial infarction or end-stage renal failure. Hypertension induces structural and functional modifications in the kidney, subsequently followed by end-stage renal disease.

The salt sensitivity plays a major role in majority of the essential hypertension. The crucial role of excess salt intake in the diet and its impact on the development and the pathogenesis of salt- sensitive hypertension have been widely acknowledged. The inability of the kidney to excrete excess salt shifts the normal pressure natriuresis relationship curve to the right. The functional as well as structural changes have been attributed to these alterations.

Lombardi et al. had shown that the salt sensitive-hypertension develops in rats after transient administration of angiotensin II. The fundamental abnormality established was focal tubulointerstitial (TI) injury. TI injury has been demonstrated to correlate with the progression of renal failure. The enhanced sodium reabsorption perceived in these circumstances may be the outcome of the local generation of vasoactive mediators in ischemic areas at sites of tubulointerstitial injury. And the peritubular capillary rarefaction observed in these models might also be responsible for the impairment .

Heme oxygenase-1 (HO-1) is a ubiquitous and redox-sensitive inducible stress protein which is the rate-limiting enzyme in the heme breakdown to generate equimolar quantities of biliverdin, free ferrous iron, and carbon monoxide (CO). Subsequently, biliverdin is rapidly converted into bilirubin by biliverdin reductase, and free iron is sequestered by ferritin. The significance of HO-1 in physiological and pathological states lies in its versatility of inducers and the protective effects exerted in condition that are associated with moderate to severe cellular stress.

Induction of HO-1 has been shown to attenuate tumour necrosis factor-α mediated cell death by retroviral delivery of human HO-1 gene into human microvessel endothelial cells. HO-1 gene up-regulation has been shown to attenuate Ang II mediated DNA damage in endothelial cells. In various animal experimental models of oxidative injury, induction of HO-1 has been demonstrated to provide protection from ongoing injury.

The relevance of HO-1 in renal diseases is further demonstrated by the presence of HO-1 protein in renal tubules in human ischemic acute tubular necrosis. The

induction of HO-1 has also been demonstrated in renal tubules in a rat model of rhabdomyolysis and nephrotoxic serum nephritis. The enhanced tubular cell HO-1 expression has been shown to provide protection against cisplatin-induced tubular cell injury.

In the present study, we evaluated the role of HO-1 in Ang II induced tubulointerstitial injury and corresponding salt-sensitive hypertension. We treated Male Sprague-Dawley rats on high salt diet (HSD) by Ang II infusion plus either hemin, an inducer of HO-1 or hemin plus zinc protoporphyrin, a HO-1 inhibitor for 2 wks, then followed for 6wks.All rats except control were infused Ang II dissolved in Ringer's lactate(R/L) via the subcutaneous implantation of an osmotic minipump. The hemin and ZnPP both were started 2 days before the pump implantation and continued for 2wks.

After 2wk all the interventions including the Ang II infusion was stopped and the osmotic pumps were removed. Rats were followed for another 6 wks before sacrifice. During whole period rats were fed high salt diet (HSD) (4% NaCl) except immediate 1wk period after pump removal in which they were fed normal salt diet (NSD) (0.4% NaCl).

Systolic BP (SBP) measurements were performed in conscious, restrained rats by tail cuff plethysmography. The BP was measured at basal period and then every week for the rest of the study.

Assessment of Renal Function was performed using the measurement of urinary protein on 0, 2, 4 and 8wks plus serum creatinine on 0, 2 and 8 wks of the study.

Histological analyses were performed by using periodic acid-Schiff and Masson Trichrome staining. Semi quantitative analyses of renal histological features were performed in a blinded manner. Tubulointerstitial injury was graded from 0 to 5, on the basis of tubular cellularity, basement membrane thickening, dilatation, atrophy or interstitial widening. Total values were expressed as tubular injury score.

Immunohistochemistry localization of HO-1, osteopontin (OPN), TGF- ß1 and rat endothelial cell antigen (RECA-1) was performed. Western Blotting was performed for HO-1 protein. Renal tissue homogenate HO activity was also determined spectrophotometrically.

The infusion of Ang II and HSD resulted in acute rise of systolic blood pressure in comparison of vehicle treated (219±4 mmHg vs. 124±2 mmHg) with elevated

serum creatinine (0.80±0.05 mg/dl vs. 0.41± 0.06) and significant proteinuria (85.3±0.7 mg/24hrs vs. 9.1±0.6mg/24hrs). Light microscopy revealed focal areas of tubular atrophy with mononuclear cell infiltration and interstitial expansion. The overexpression of osteopontin and TGF-ß1 accompanied by diminished expression of rat endothelial cell antigen-1, the hallmarks of TI injury were observed. At 2 wk, all interventions were withdrawn and SBP returned towards normal. After brief normal salt diet, rats were again placed on HSD, resulting in progressive increase in SBP in HO-1 inhibited group.

In contrast hemin, a potent inducer of HO-1, treated group showed significant attenuation of Ang II induced HTN (123±6 mmHg) with improved renal functions. Furthermore, hemin treated group showed no significant tendency of increase in blood pressure despite reintroduction of HSD. Histological parameters and immunohistochemical markers were significantly improved by the induction of HO-1.

To further confirm the implication of HO-1 in the BP lowering effect of hemin and its role in salt-sensitive HTN, we inhibited the activity of HO by ZnPP, a non-selective inhibitor of HO activity. Inhibition of HO activity resulted in exaggerated BP (225±4.3 mmHg) after Ang II infusion and salt-sensitivity as represented by tendency towards increase in BP after reinitiation of HSD.

Our study demonstrate that short term, continuous infusion of Ang II in pressor doses with high salt diet in the SD rats result into focal tubulointerstitial injury and the development of salt-sensitive hypertension . We also showed that induction of inducible HO-1 in renal tubules after the administration of hemin, which augment HO-1, ameliorates focal tubulointerstitial injury and attenuates salt-sensitive hypertension. Renal function also showed amelioration of Ang II induced deterioration in HO-1 upregulated group. Histological finding revealed that HO-1 upregulation significantly ameliorated Ang II induced focal tubulointerstitial injury including microvascular alterations. Immunohistochemistry showed that Ang II also induced HO-1 expression however hemin induced HO-1 expression was more extensively distributed. Moreover, HO-1 significantly downregulated oxidative markers such as osteopontin and profibrogenic molecule TGF-ß1 and significantly prevented dissolution of lacy peritubular capillary network as seen by RECA-1 immunostaining.

The several lines of evidence suggest that the administration of the HO-1 inducer hemin ameliorated and non-selective HO inhibitor ZnPP exacerbated renal injury induced by Ang II infusion and high salt diet.

We also observed that hemin untreated group develop high blood pressure after reinitiation of high salt diet, in contrast there was no increase in hemin treated group suggesting that up regulation of HO-1 ameliorates tubulointerstitial injury including microvascular changes which led to significant attenuation of salt-sensitivity and resultant HTN.

These finding strongly suggest that initial Ang II and HSD induced focal tubulointerstitial injuries, which may be the consequence of ischemia secondary to peritubular capillary injury are the primary lesions which ultimately lead to the salt sensitivity and development of hypertension.

The precise renal protective mechanisms behind these findings are yet to be elucidated. However numerous studies have documented the cyto-protective and anti-oxidant effect of HO-1. For example induction of the HO-1 in the kidney and the other tissues exposed to various types of injuries confers several effective mechanism such as detoxification of pro-oxidant heme, safe sequestration of free iron liberated during heme degradation by ferritin, which is coinduced with HO-1, the production of carbon mono-oxide exerting antiapoptotic, anti-inflammatory , vasodilatory effects and the generation of anti-oxidant metabolites such as bilirubin which scavenge peroxy radicals and inhibit activation of NADPH oxidase and protein kinase C, the key signalling steps in oxidant–induced vascular injury. Recently, Taylor et al has demonstrated the significant relationship among oxidative stress, vascular injury and the salt-sensitive HTN by showing that NADPH oxidase dependent elevation in ROS contributes to salt-sensitive HTN. In other study, CO-releasing molecule (CO-RM) considerably reduced the levels of tumour necrosis factor-alpha, another mediator of the inflammatory response.

Studies in knockout mice and HO-1 deficiency in human indicates association between HO-1 deficiency and susceptibility to oxidative stress with an increased pro-inflammatory state and severe endothelial damage suggesting that with its metabolites HO-1 is a strong anti-inflammatory mediator in vivo. HO-1 upregulation also inhibits microvascular endothelial cell-leukocyte adhesion through the action of bilirubin. Overexpression of HO-1 led to an increase in capillary sprouting and showed that HO activity up-regulates VEGF production and augments the capability

of endothelial cells to respond to exogenous stimulation. Knock out of HO-1 gene impaired induction of VEGF by hemin, confirming the involvement of HO-1 in regulation of angiogenesis. These previous findings strongly support our observation, showing preservation of lacy peritubular capillary network in HO-1 upregulated group. Furthermore, it has been shown that HO system participates in the regulation of salt and water excretion through diuresis and natriuresis. And there might be a role of HO system in the modulation of epithelial sodium channel (ENaC) and serum-glucocorticoid-inducible kinase 1 (SGK1), which needs to be elucidated.

In summary, the short term, continuous infusion of Ang II and HSD induced tubulointerstitial injury leads to salt sensitivity and HTN. The underlying Ang II induced TI injury and resulting salt-sensitive HTN was significantly attenuated by upregulation of HO-1 through hemin treatment in the rat kidney. Our result suggest a central role of HO-1 as a protective agent against Ang II and HSD induced renal injury and demonstrate that pharmacological induction of HO-1 might be of clinical significance.

神戸大学大学院医学系研究科（博士課程）

論文審査の結果の要旨			
受付番号	甲 第 1793 号	氏　名	ASHOK PRADHAN
論文題目 Title of Dissertation	Heme-oxygenase upregulation ameliorates angiotensin II induced tubulointerstitial injury and salt-sensitive hypertension ヘムオキシゲナーゼ発現増強は、アンギオテンシンIIによる尿細管間質病変と食塩感受性高血圧を軽減する		
審査委員 Examiner	主　査 Chief Examiner 掛山正宏 副　査 Vice-examiner 秋田穂束 副　査 Vice-examiner 猪野滋一		
審査修了日	平成19年3月7日		

（要旨は 1,000 字ー2,000 字程度）

Hypertension (HTN) is a multi-factorial disease and the leading risk factor for various cardiovascular and renal complications such as stroke, myocardial infarction or end-stage renal failure. Hypertension induces structural and functional modifications in the kidney, subsequently followed by end-stage renal disease.

The salt sensitivity plays a major role in majority of the essential hypertension. The crucial role of excess salt intake in the diet and its impact on the development and the pathogenesis of salt- sensitive hypertension have been widely acknowledged. The inability of the kidney to excrete excess salt shifts the normal pressure natriuresis relationship curve to the right. The functional as well as structural changes have been attributed to these alterations.

Lombardi et al. had shown that the salt sensitive-hypertension develops in rats after transient administration of angiotensin II. The fundamental abnormality established was focal tubulointerstitial (TI) injury. TI injury has been demonstrated to correlate with the progression of renal failure. The enhanced sodium reabsorption perceived in these circumstances may be the outcome of the local generation of vasoactive mediators in ischemic areas at sites of tubulointerstitial injury. And the peritubular capillary rarefaction observed in these models might also be responsible for the impairment .

Heme oxygenase-1 (HO-1) is a ubiquitous and redox-sensitive inducible stress protein which is the rate-limiting enzyme in the heme breakdown to generate equimolar quantities of biliverdin, free ferrous iron, and carbon monoxide (CO). Subsequently, biliverdin is rapidly converted into bilirubin by biliverdin reductase, and free iron is sequestered by ferritin. The significance of HO-1 in physiological and pathological states lies in its versatility of inducers and the protective effects exerted in condition that are associated with moderate to severe cellular stress.

In the present study, we evaluated the role of HO-1 in Ang II induced tubulointerstitial injury and corresponding salt-sensitive hypertension. We treated Male Sprague-Dawley rats on high salt diet (HSD) by Ang II infusion plus either hemin, an inducer of HO-1 or hemin plus zinc protoporphyrin (Zn PP), a HO-1 inhibitor for 2 wks, then followed for 6wks.All rats except control were infused Ang II dissolved in Ringer's lactate(R/L) via the subcutaneous implantation of an osmotic minipump. The hemin and ZnPP both were started 2 days before the pump implantation and continued for 2wks.

After 2wk all the interventions including the Ang II infusion was stopped and the osmotic pumps were removed. Rats were followed for another 6 wks before sacrifice. During whole period rats were fed high salt diet (HSD) (4% NaCl) except immediate 1wk period after pump removal in which they were fed normal salt diet (NSD) (0.4% NaCl).

Systolic BP (SBP) measurements were performed in conscious, restrained rats by tail cuff plethysmography. The BP was measured at basal period and then every week for the rest of the study.

Assessment of renal function was performed using the measurement of urinary protein on 0, 2, 4 and 8wks plus serum creatinine on 0, 2 and 8 wks of the study.

Histological analyses were performed by using periodic acid-Schiff and Masson Trichrome staining. Semi quantitative analyses of renal histological features were performed in a blinded manner. Tubulointerstitial injury was graded from 0 to 5, on the basis of tubular cellularity, basement membrane thickening, dilatation, atrophy or interstitial widening. Total values were expressed as tubular injury score.

Immunohistochemistry localization of HO-1, osteopontin (OPN), TGF- ß1 and rat endothelial cell antigen (RECA-1) was performed. Western Blotting was performed for HO-1 protein. Renal tissue homogenate HO activity was also determined spectrophotometrically.

The infusion of Ang II and HSD resulted in acute rise of systolic blood pressure in comparison of vehicle treated with elevated serum creatinine and significant proteinuria. Light microscopy revealed focal areas of tubular atrophy with mononuclear cell infiltration and interstitial expansion. The overexpression of osteopontin and TGF-ß1 accompanied by diminished expression of rat endothelial cell antigen-1, the hallmarks of TI injury were observed. At 2 wk, all interventions were withdrawn and SBP returned towards normal. After brief normal salt diet, rats were again placed on HSD, resulting in progressive increase in SBP in HO-1 inhibited group.

In contrast hemin treated group showed significant attenuation of Ang II induced HTN with improved renal functions. Furthermore, hemin treated group showed no significant tendency of increase in blood pressure despite reintroduction of HSD. To further confirm the implication of HO-1 in the BP lowering effect of hemin and its role in salt-sensitive HTN, we inhibited the activity of HO by ZnPP. Inhibition of HO activity resulted in exaggerated BP after Ang II infusion and salt-sensitivity as

represented by tendency towards increase in BP after reinitiation of HSD. Histological parameters and histochemical markers were significantly improved by the induction of HO-1.

Our study demonstrate that short term, continuous infusion of Ang II in pressor doses with high salt diet in the SD rats result into focal tubulointerstitial injury and the development of salt-sensitive hypertension . We also showed that induction of inducible HO-1 in renal tubules after the administration of hemin, which augment HO-1, ameliorates focal tubulointerstitial injury and attenuates salt-sensitive hypertension. Renal function also showed amelioration of Ang II induced detoriation in HO-1 upregulated group. Histological finding revealed that HO-1 upregulation significantly ameliorated Ang II induced focal tubulointerstitial injury including microvascular alterations. Immunohistochemistry showed that Ang II also induced HO-1 expression, however hemin induced HO-1 expression was more extensively distributed. Moreover, HO-1 significantly downregulated oxidative markers such as osteopontin and profibrogenic molecule TGF-ß1 and significantly prevented dissolution of lacy peritubular capillary network as seen by RECA-1 immunostaining.

The several lines of evidence suggest that the administration of the HO-1 inducer hemin ameliorated and non-selective HO inhibitor ZnPP exacerbated renal injury induced by Ang II infusion and high salt diet.

We also observed that hemin untreated group develop high blood pressure after reinitiation of high salt diet, in contrast there was no increase in hemin treated group suggesting that upregulation of HO-1 ameliorates tubulointerstitial injury including microvascular changes which led to significant attenuation of salt-sensitivity and resultant HTN.

These finding strongly suggest that initial Ang II and HSD induced focal tubulointerstitial injuries, which may be the consequence of ischemia secondary to peritubular capillary injury are the primary lesions which ultimately lead to the salt sensitivity and development of hypertension.

The precise renal protective mechanisms for cyto-protective and anti-oxidant effect of HO-1may include detoxification of pro-oxidant heme, safe sequestration of free iron liberated during heme degradation by ferritin, which is coinduced with HO-1, the production of carbon mono-oxide exerting antiapoptotic, anti-inflammatory , vasodilatory effects and the generation of anti-oxidant metabolites such as bilirubin

which scavenge peroxy radicals and inhibit activation of NADPH oxidase and protein kinase C, the key signalling steps in oxidant–induced vascular injury. HO-1 upregulation also inhibits microvascular endothelial cell-leukocyte adhesion through the action of bilirubin. HO activity up-regulates VEGF production and angiogenesis. These previous findings strongly support our observation, showing preservation of lacy peritubular capillary network in HO-1 upregulated group. Furthermore, it has been shown that HO system participates in the regulation of salt and water excretion through diuresis and natriuresis. And there might be a role of HO system in the modulation of epithelial sodium channel (ENaC) and serum-glucocorticoid-inducible kinase 1 (SGK1), which needs to be elucidated.

In summary, the short term, continuous infusion of Ang II and HSD induced tubulointerstitial injury leads to salt sensitivity and HTN. The underlying Ang II induced TI injury and resulting salt-sensitive HTN was significantly attenuated by upregulation of HO-1 through hemin treatment in the rat kidney. Our result suggest a central role of HO-1 as a protective agent against Ang II and HSD induced renal injury and demonstrate that pharmacological induction of HO-1 might be of clinical significance. The candidate, having completed studies on the protective role of HO-1 in Angiotensin II induced tubulointerstitial injury and salt-sensitive hypertension and having advanced the field of knowledge in the area of hypertension and kidney is hereby recognized as having qualified for the degree of Ph.D.(Medicine).