博士論文（要約）

Relationship between Cyclooxygenase1, Cyclooxygenase2 and Chronic Kidney Disease

（シクロオキシゲナーゼの慢性腎臓病に与える影響）

レヘマン　ラタパティ

熱合曼　　拉塔怕提

Reheman　Latapati

Abstract

Background:

Cyclooxygenase (COX), a rate-limiting enzyme in the prostaglandin (PG) biosynthesis pathway exists in two major isoforms: constitutive COX1 and inducible COX2. They have similar amino acid sequence and enzymatic function, although their physiological functions and cellular localization are entirely different. COX1 expressed in most tissues, while COX2 is induced by inflammatory stimulation. Prostaglandins are generated by the conversion of free arachidonic acid to intermediate prostaglandin H2 (PGH2) by COX. Afterwards, PGH2 is metabolized to more stable biologically active prostanoids, including prostaglandin E2 (PGE2), prostacyclin I2 (PGI2), prostaglandin F2α, prostaglandin D2, and thromboxane A2 (TxA2) by the synthases PGES, PGIS, PGDS, PGFS and TXS respectively. Prostaglandin E2 (PGE2) is one of the most abundant prostanoid in the kidney and plays a role in promoting renal fibrosis. Until to now three kind of PGE synthases have been identified; these are microsomal PGE synthase 1 (mPGES-1), microsomal PGE synthase 2 (mPGES-2), and cytosolic PGE synthase (cPGES). It is well recognized that PGE2 mainly generated by mPGES-1.

Former reports indicated that overproduction of PGE2 has been shown to be associated with increased renal hypertrophy, hyperfiltration and urine protein in streptozotocin induce diabetic model, also PGE2 excretion increased in angiotensin II stimulation induced kidney failure model. In contrast, high-salt (HS) loading, in which renin-angiotensin axis is suppressed, also increases the expression of COX2/ mPGES1/ PGE2 in the renal medulla. The mechanism of those enzymes regulation and increment in PGE2 under high-salt loading and its relation to organ damages remains poorly understood. Salt loading leads wide variety of responses such as inducing oxidative stress, the mineralocorticoid receptor (MR) activation, and inflammation and so on. In the present study we investigated the role of ROS and MR on high-salt-induced COX2/ mPGES1/ PGE2 expression levels.

Method:

Three-weeks-old Sprague-Dawley (SD) rats were subjected to left uninephrectomy and subsequently divided into the following groups: (i) normal-salt (NS; 0.3% NaCl) diet 2 weeks and (ii) 4 weeks; (iii) high-salt (HS; 8% NaCl) diet for 2 weeks and (iv) HS diet for 4 weeks; (v) HS diet plus the antioxidant superoxide dismutase mimetic 4-hydroxy-2,2,6,6-tetramethyl-piperidine-*N*-oxyl, Tempol (0.6 mmol/kg/day in drinking water) 2 weeks and (vi) HS plus tempol 4 weeks; (vii) HS diet plus treatment with the MR blocker eplerenone (Ep; 100 mg/kg/day in chow) for 4 weeks. Body weight, blood pressure, renal medulla mPGES-1, COX1, COX2 levels, urinary PGE2 excretion and urinary isoprostane were evaluated and compared with pathological changes as well as renal damage markers Col1a1, Col3a1 and oxidative stress level. The effects of the ROS inhibitor tempol and MR blocker eplerenone were examined.

Results:

Hypertension and proteinuria were developed at early stage of 2 weeks high salt loading (SBP: 110 ± 2.6 vs. 152 ± 11mmHg, protein: 1.57 ± 0.3 vs. 75.7 ± 21 mg/ day) and aggravated after 4 weeks HS loading (SBP: 130 ± 3.0 vs. 194 ± 7.0mmHg, protein: 10.4 ± 2 vs. 120 ± 28 mg/day), this data strongly show the CKD model was successful. In histological study kidney section stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS) and Azan staining showed glomerulotubulointerstitial fibrosis and accumulation of matrix deposition. I also studied mPGES-1 mRNA and protein levels after high salt loading these parameters elevated in 2 and 4 weeks group compared with the normal salt groups. Oxidative stress markers isoprostane and urinary PGE2 level partly increased in high salt 2 weeks loaded group, and in 4 weeks high salt loaded group significantly increased compared with respective controls. These changes were markedly ameliorated by ROS inhibitor tempol treatment independent from blood pressure, but MR blocker eplerenone failed to reverse mPGES-1 mRNA and protein, urinary PGE2 and isoprostane level even though reduced blood pressure and reversed kidney function.

Conclusion:

Although both tempol and eplerenone reversed renal damage, only tempol can reduce COX2/ mPGES-1/ PGE2 level. From these data, we found two new regulatory mechanisms in PGE2. Firstly, high salt activated ROS may responsible for over expression of PGE2. Secondly, PGE2 not regulated by either intrarenal blood pressure or MR.