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学位論文題目	Novel Therapeutic Role for Dipeptidyl Peptidase III in the Treatment of Hypertension （高血圧に対する新規治療としてのジペプチジルペプチダーゼ III の役割）
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別紙様式３（課程博士・論文博士共用）

論　文　内　容　要　旨

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学位論文題目	Novel therapeutic role for dipeptidyl peptidase III in the treatment of hypertension. （高血圧に対する新規治療としてのジペプチジルペプチダーゼ III の役割）		

目的

Hypertension is one of the major metabolic syndromes, and usually causes lethal cardiovascular diseases, such as myocardial or cerebral infraction. Although there are many drugs available for treatment of hypertension, substantial population of patients has still difficulty for controlling blood pressure. Angiotensin II (Ang II) plays an essential role in the progression of hypertension. Thus, Ang II could be a main target for exploring novel anti-hypertensive therapeutics.

Dipeptidyl peptidase III (DPP III, EC 3.4.14.4) was reported to cleave Ang II (Lee CM, et al. *J Biol Chem*. 1982). However, little had been understood for the enzymatic property of DPP III for Ang II cleavage, and for the *in vivo* activity of DPP III. The purposes of the applicant on this study were to investigate these unrevealed issues, potential use of DPP III in hypertension, and its protective effects on hypertension-sensitive organs, such as the heart and kidney.

方法

First, we generated and purified the recombinant protein of DPP III from the lysates of *E. coli* transformed with pGEX-DPP III plasmid. The enzymatic activities and properties of DPP III against Ang II were examined and confirmed by reversed-phase liquid chromatography and mass spectrometry. C57BL/6J mice, 8-10 weeks of age, were used as a hypertensive mouse model by subcutaneously implanting a micro-osmotic pump containing Ang II (400 ng/kg/min) or noradrenalin (4 μg/kg/min) for 2-4 weeks. Blood pressure was measured in conscious acclimatized mice using a tail cuff method. DPP III, an angiotensin receptor blocker candesartan, or saline were injected via the tail vein. Prior to injection, blood pressure elevation by Ang II-infusion was confirmed (systolic blood pressure ≥130 mmHg). Ang II concentration in plasma was measured with the Angiotensin II ELISA kit according to the manufacturer's protocol. Histological analyses were performed for paraffin-embedded samples from hearts and kidneys. Cardiac function was evaluated by echocardiography. Transmission electron microscopy observation was employed for morphological analysis of kidney podocytes. To assess kidney injury, RT-qPCR for inflammatory markers and SDS-PAGE followed by CBB staining for detection of urinary albumin were performed.

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等を用いて印字すること。

２．※印の欄には記入しないこと。

結果

We finely determined the enzymatic activity of DPP III for Ang II digestion with $K_m = 3.7 \times 10^{-6}$ mol/L and $V_{max} = 3.3 \times 10^{-9}$ mol/L/sec. In the *in vivo* experiments, DPP III remarkably reduced blood pressure in Ang II-infused hypertensive mice without alteration of heart rate. Serum Ang II concentration was actually decreased by DPP III administration. DPP III did not affect hemodynamics in noradrenalin-induced hypertensive mice or normotensive mice, suggesting its specificity for Ang II. When DPP III was intravenously injected every other day for 4 weeks just after Ang II micro-osmotic implantation in mice, Ang II-induced cardiac fibrosis and hypertrophy were significantly attenuated. Furthermore, administration of DPP III dramatically reduced the increase in urine albumin excretion, and kidney injury and inflammation markers caused by Ang II infusion without tissue morphological changes in the kidney. These DPP III effects were similar to that observed by administration of candesartan.

考察

In *in vitro* analysis, the K_m and V_{max} values for DPP III-cleaved Ang II to Ang IV shown above are different from a previous report. Because the reaction mixture in the previous report was indicated to technically contain contaminated peptides derived from the rat brain during the purification process, such peptides could act as uncompetitive inhibitors to lower K_m and V_{max}. In the *in vivo* experiments, we showed that administration of DPP III dramatically lowered blood pressure in Ang II-infused hypertensive mice, suggesting the potential of DPP III as an anti-hypertensive drug. We also showed that DPP III had cardioprotective effects by preventing cardiac hypertrophy and fibrosis, and that DPP III administration showed inhibition in the albumin excretion in Ang II-infused mice. These results were mediated by direct degradation of Ang II by DPP III, and provide a novel insight that the mode of inhibition of the renin-angiotensin II system (RAS) by DPP III, which is different from that by any conventional anti-hypertensive drugs. This novel mechanism for inhibition of the RAS by DPP III appears to contribute to the protection from Ang II-initiated cardiac and renal damages.

結論

The *in vitro* enzymatic activity against Ang II and *in vivo* pressure-lowering effect of DPP III were precisely characterized in this study. DPP III showed tissue protection in the heart and kidney to a similar extent as candesartan in Ang II-infused hypertensive mice. Because DPP III is endogenously produced in humans, its administration is considered to be relatively safe. Taken together, the results demonstrated in this study suggest a new therapy for hypertension, focusing on DPP III.

別紙様式８（課程博士・論文博士共用）

学位論文審査の結果の要旨

整理番号	787	氏　　名	逄　暁玲
論文審査委員			

（学位論文審査の結果の要旨）※明朝体１１ポイント、６００字以内で作成のこと

アンジオテンシンⅡ（Ang Ⅱ）は高血圧発症の鍵分子であるが、その分解に関与しているジペプチジルペプチダーゼⅢ（DPP Ⅲ）の役割については不明の点が多かった。そこで申請者は、DPP3の生理的役割と高血圧治療薬としての可能性を検討するために、*in vitro* 及び *in vivo* の両側面から、Ang Ⅱに及ぼす影響、血圧変動と臓器保護効果について検討し、以下の点を明らかにした。

1）DPP ⅢはAng Ⅱを Km=3.7 μmol/L、Vmax=3.3 nmol/L/sec で分解した。
2）Ang Ⅱ誘発高血圧マウスにおいて、DPP Ⅲは心拍数に影響を及ぼさず、血圧と血中Ang Ⅱ濃度を低下させた。
3）DPP Ⅲは、ノルアドレナリン誘発生高血圧に影響を及ぼさなかった。
4）DPP Ⅲは、Ang Ⅱによって引き起こされる心筋繊維症や心肥大を抑制した。
5）DPP Ⅲは、Ang Ⅱによって引き起こされる、尿中アルブミン排泄量の増加など腎機能障害を抑制した。
6）上記の効果は、Ang Ⅱ受容体拮抗薬であるカンデサルタンと類似していた。

本論文は、DPP Ⅲの高血圧治療薬に関する新規治療薬の可能性について新しい知見を与えたものであり、最終試験として論文内容に関連した試問を受け合格したので、博士（医学）の学位論文に値するものと認められた。

（総字数 545 字）

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