Identification of angiotensin peptides modulating the harmful effects of Ang II in CKD

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BACKGROUND

The family of angiotensin peptides has been steadily growing in recent years. Most are fragments of angiotensin II (Ang-II) with different affinities to the known angiotensin receptors. Here we describe a novel endogenous Ang-II like octapeptide in plasma from healthy humans and end-stage renal failure patients, which acts as a stronger agonist at MAS receptors than Ang 1-7.

METHODS

We directly fractionated the human plasma by size-exclusion chromatography. We equilibrated the size-exclusion chromatography gel with 0.9% NaCl in water. We loaded the eluate of the size-exclusion chromatography onto a monolithic reverse-phase chromatography column. The lyophilized fractions from the reverse-phase chromatography were analyzed by MALDI mass spectrometry (MALDIMS) and MALDI-time of flight (TOF/TOF) fragment ion analysis. The human genome database of the National Center for Biotechnology Information was searched using the BLAST algorithm for genes coding the amino acid sequence of angioprotectin. Angioprotectin was synthesized automatically by the solid-phase method using standard Fmoc chemistry in continuous flow mode. Contractile effects of vasoconstriction induced by angioprotectin and Ang II were assayed on rings of isolated rat aorta.

RESULTS

Figure 1A shows the chromatographic purification and Figure 3 shows a structural analysis by matrix-assisted laser desorption /ionisation time-of-flight/time-of-flight (MALDI-TOF/TOF) revealed an angiotensin octapeptide with the sequence Pro-Glu-Val-Tyr-Ile-His-Pro–Phe, which differs from Ang II in Pro^1 and Glu^2 instead of Asp^1 and Arg^2, in the following named Angioprotectin (Figure 1B and C).

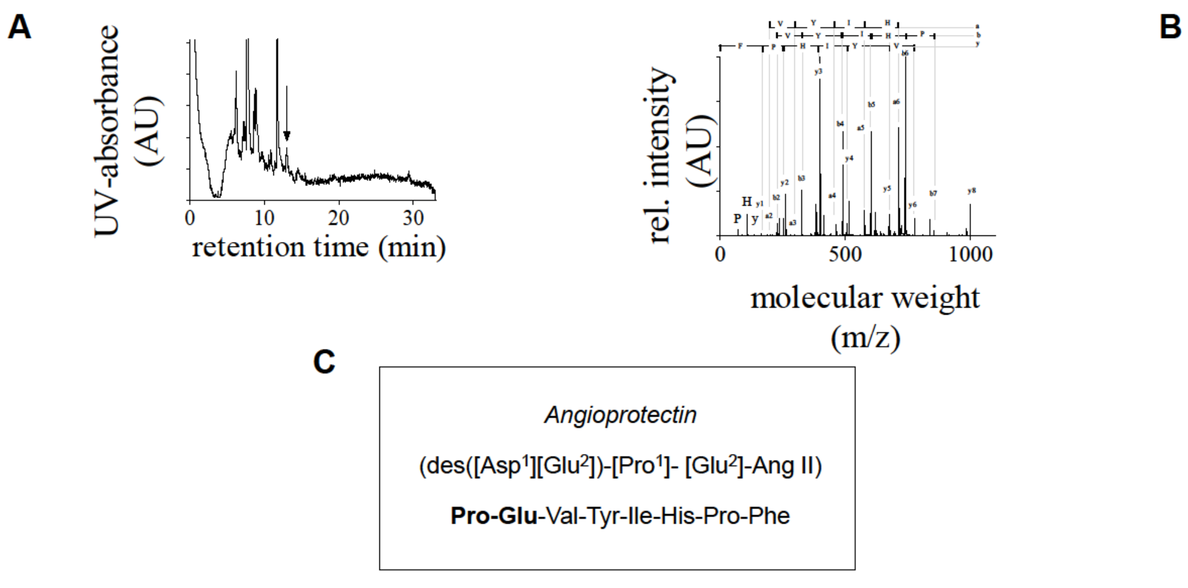

Figure 1: (**A**) Chromatogram of an analytic reverse-phase HPLC of the fraction with strong vasoconstrictive properties on testing in the isolated perfused rat kidney. (Chromolith RP, eluent A: 0.1 % trifluoroacetic acid (TFA) in water; eluent B: 0.1% TFA in water/acetonitrile; (**B**) Matrix-assisted-laser-desorption/ionisa-tion TOF/TOF-mass spectrum of the vasoregulatoric peptide. (**C**) Amino acid sequence of Angioprotectin (bold: difference from the amino acid sequence of angiotensin II).

Angioprotectin antagonized the contractile actions of Ang-II on rat aortic rings. Equimolar Angioprotectin concentrations reduced Ang-II-induced constrictions by about 30 % (Figure 2).

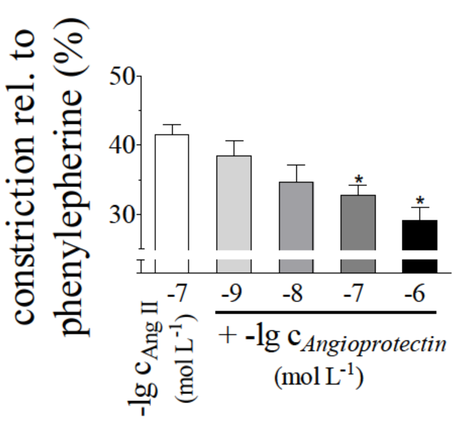

Figure 2: Contractions of rings of isolated rat aorta induced by Ang-II in the absence of increasing concentrations of Angioprotectin

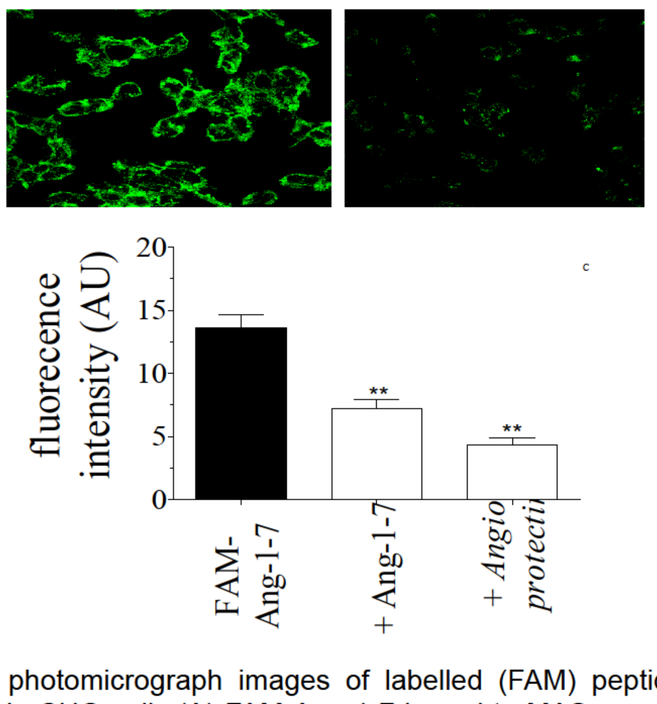

Figure 3: Confocal photomicrograph images of labelled (FAM) peptides bound to MAS receptors expressed in CHO cells (A) FAM-Ang-1-7 bound to MAS receptors; (B) FAM-Ang-1-7 bound to MAS receptors in the presence of Angioprotectin; (C) Quantification of displacement effect

Fluorescently labelled (FAM) Ang-1-7 peptide bound to MAS receptors (Figure 3). Angioprotectin displaced the substrate of the MAS receptor. The displacement effect of Angioprotectin is stronger than the effect of Ang-1-7. Angioprotectin concentrations in plasma were determined by MALDI-TOF/TOF-MS, because conventional enzyme immunoassay for Ang II quantification did not distinguish between Ang II and Ang A (Figure 4).

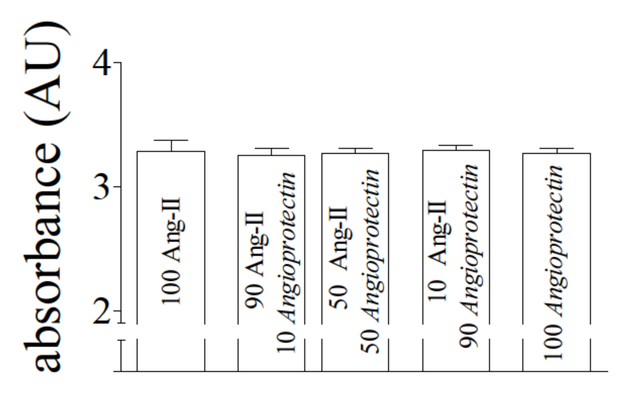

Figure 4: Quantification of Angio-protectin and Ang II by enzyme immunoassay (in pmol/L).

In ESRD patients, Angioprotectin plasma concentrations increased up to five-fold compared to healthy control subjects. Ang-II concentrations did not differ in healthy subjects and end-stage renal failure patients (Figure 5).

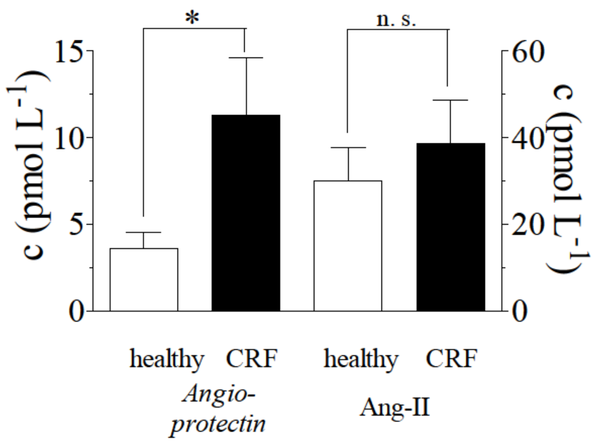

Figure 5: Mass-spectrometry-based determination of Angioprotectin (left Y-axis) and Ang-II (right Y-axis) levels in plasma of healthy control subjects (open bars) and ESRD patients (black bars).

CONCLUSIONS

In conclusion, our findings show that

(1) human plasma contains a vasodilatory octapeptide Angioprotectin

(2) plasma concentrations of Angioprotectin are increased about 3-fold in ESRD

(4) immunoassay did not discriminate between Angioprotectin and Ang-II.

(5) Angioprotectin may modulate pathological Ang-II effects

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