International Journal of Cardiology 148 (2011) 76–84

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

A vaccine for hypertension based on peptide AngI-R: A pilot study

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ARTICLE INFO

Article history:
Received 10 April 2009
Received in revised form 8 August 2009
Accepted 18 October 2009
Available online 24 November 2009

Keywords:
Anti-hypertensive
Angiotensin-converting enzyme inhibitory
Peptide
Immunization

ABSTRACT

Aims: We aim to modulate the renin–angiotensin system (RAS) by active immunization against angiotensin I/II, potentially by a novel type of peptide—'AngI-R' for the treatment of hypertension.
Method: 27 spontaneous hypertensive rats (SHRs) were randomly divided into 3 groups ($n=9$). Effect of AngI-R on systolic blood pressure of SHRs was determined by its subcutaneous injection into SHRs with anti-angiotensin peptide AngI-R, captopril, physiological salt solution in the same schedule. Systolic blood pressure was monitored by tail-cuff arterial blood pressure measurement method and then calculated from the average of three measurements in each animal at a desired time (0, 4, 8, 16W). AngI, AngII and anti-AngI antibody were detected by ELISA and western blotting method. At the end, the pathologic changes in different organs of SHRs were observed.
Result: The results exhibited a marked effect to lower the systolic blood pressure (−15 mm Hg) of SHRs after subcutaneous injection with peptide AngI-R. The anti-hypertensive effect lasted for over 1 month. The level of AngI and AngII in the vaccinated group, after administration of AngI-R was lower than the one in the control group and the pre-administered group. The anti-AngI/II antibody titre in the experiment group significantly increased.
Conclusion: The peptide AngI-R was able to decrease the blood pressure of SHRs. The peptide AngI-R induced an immune response to inhibit AngI and AngII, resulting in the decrease in blood pressure in SHRs.

1. Introduction

The renin–angiotensin system (RAS) possesses an important physiological role, influencing normal cardiovascular status and contributing to diseases such as hypertension and heart failure [8]. The RAS has been extensively studied and AngII activation has been nailed as the culprit for most of the cardiovascular functional and structural deterioration [20]. The RAS has thus been a target for clinical control, either by ACE inhibitors [4], or AngII-receptor antagonists (ARB) [14]. Despite the fact that these drugs are highly effective, immunization against AngI has been suggested as an attractive alternative for RAS-targeted control of hypertension [15,20] due to undesirable side-effects [9,22] and poor oral drug compliance. Besides these clinical advantages potential benefits include avoiding compensation of chymase, improvements in compliance management, smooth and progressive onset of action for patients with mild hypertension or left ventricular dysfunction, and so on.

An AngI vaccine induces immunoglobulins against AngI hormone, preventing generation of AngII and subsequently preventing the increase in blood pressure. Similar methods of inducing immunoglobulins for a number of biological and clinical applications have been documented previously. [1,2,5,7,10,12–14,19,20,23]In this paper we describe our study on a new conjugate vaccine containing a peptide immunogen 'AngI-R'. The peptide was administered to SHRs for immunization and blood pressure was assessed on the SHRs.

2. Materials and method

2.1. AngI-R peptide synthesis

We modified peptide AngI-R according to the structure of Ang I. The structure of Ang I was modified with amino acids at its C-terminus in order to prevent AngI-R from proteolytic cleavage. In such a way, the biological activity of Ang I would be removed, but retain their immunogenicity to prepare the vaccine. Peptide AngI-R was synthesized by using the solid phase method with a SYMPHONY 12-channel solid phase peptide synthesizer (Applied Protein Technologies Inc., Software Version.201 USA). The synthetic peptide was purified by using Shimadzu HPLC (Shimadzu Inc, Software Class-VP. Sevial System, USA). It contained ten amino acids (Asp–Arg–Val–Tyr–IIe–His–Pro–Phe–Sar–Leu) with a molecular weight of 1230.5 kDa. The physical property is a white powder. The peptide was conjugated to bovine serum albumin (BSA) at its N-terminus.

2.2. In vitro stability of AngI-R against ACE

Angiotensin-converting enzyme from rabbit lung (EC 3.4.15.1) was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). The synthetic peptide angiotensinI-human was purchased from AnaSpec, Inc. (San Jose, CA, USA). Synthetic AngI-R peptide was

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doi:10.1016/j.ijcard.2009.10.027

synthesized by HD Bioscience Corporation (Shanghai, China), its purity was measured by HPLC and the structure was verified by mass spectrometry. The high performance liquid chromatographic system (HPLC) Agilent 1100 (Applied Biosystems, Foster City, CA, USA) was processed with Pepmap C 18 analytical column (250×4.6 mm I.D., 5 um, 300A) at a flow rate of 0.8 ml/min. Buffer A was 0.1% trifluroacetic acid (TFA) in water and buffer B was 0.1% TFA in acetonitrile (ACN). The mass spectrometry analysis of the samples was performed on an API 2000 Q-trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) in the scan type of Enhanced MS. The apparatus was equipped with a Turbo Ion Spray source and operated in the positive ionization mode.

In order to determine the ACE hydrolysis of AngI-R and AngI, an HPLC assay was developed. Desired assays were prepared in a reaction volume of 600 ul in 0.05 M Tris–HCL Buffer containing 0.3 NaCl at PH 7.8. Assays prepared consisted of; *no enzyme* AngI control consisted of 21 uM of Ang, positive AngI control consisted of 21 uM of AngI and 5 mIU of ACE, AngI-R control consisted of 21 uM of AngI-R, AngI-R test assay consisted of 21 uM of AngI-R and 5 mIU of ACE. Reactions were allowed by incubating at 37 °C for 30 min and stopped by addition of 300 ul of 0.2 M HCL. The aliquot of the incubation mixture was centrifugated at 12,000 rpm for 10 min at room temperature. The supernatant was subjected into a pre-equilibrated Pepmap C18 analytical column at a

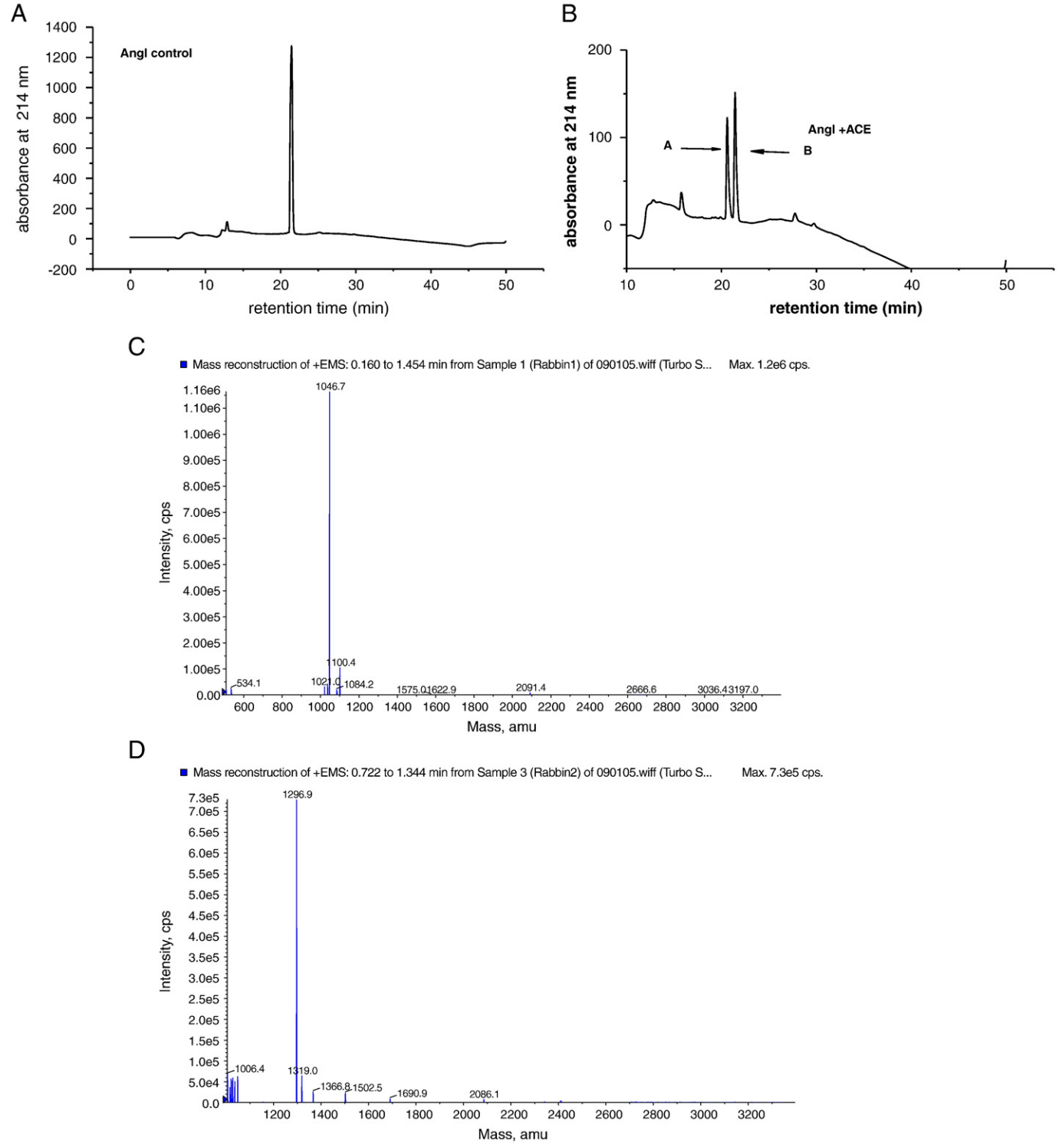

Fig. 1. ACE cleaves AngI (decapeptide, m/z 1296) to AngII (octapeptide, m/z 1046). AngI (21 uM) was incubated with ACE (5 mIU) or no enzyme, as indicated, for 30 min at 37 °C and the products were analyzed by HPLC and mass spectrometry. (A) AngI control without ACE by HPLC. (B) AngI with ACE analyzed by HPLC showing degradation products indicated as peak A and peak B. (C) Peak A in B analyzed by mass spectrometry showing a molecular wt (m/z) of 1046 (AngII). (D) Peak B in B analyzed by mass spectrometry showing the non cleaved AngI (m/z 1296).