ACE2 activity is increased in monocyte-derived macrophages from prehypertensive subjects

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Abstract

Background. Hypertension is a major risk factor for cardiovascular disease and the renin–angiotensin–aldosterone system (RAAS) plays a central pathophysiological role in its formation. Angiotensin-converting enzyme (ACE) and its homologue ACE2 control the formation of counteracting effectors, angiotensin II (AngII), a potent vasopressor and Ang-(1–7) which has vasodilatory action. It is therefore hypothesized that the balance of the activities of these two enzymes, ACE and ACE2, could be important for the control of blood pressure (BP).

Methods. Monocyte-derived macrophages were isolated from blood samples of normotensives (NT), prehypertensives (preHTN) and untreated hypertensive (HTN) male patients (n = 28, 18 and 11, respectively). The activities of ACE2 were determined by measuring leucine or phenylalanine released following hydrolysis of Ang I and Ang II, respectively. The activity of ACE was measured using a synthetic substrate.

Results. The levels of BP were 112.6 ± 1.4/74.8 ± 1.2, 128.3 ± 0.8/78.1 ± 1.2 and 151.4 ± 2.7/99.3 ± 2.4 mmHg in the NT, preHTN and HTN, respectively (P < 0.001). The ACE2-mediated Ang II degrading activity (ACE2-II) was 1201 ± 241 fmol/min/mg cell protein in NT subjects and was significantly (P < 0.01) increased by 2.4-fold in preHTN. ACE2-II activity in HTN and NT was not significantly different. ACE2-mediated Ang I hydrolysis (ACE2-I) was 85-fold lower than the ACE2-II activity.

ACE activity in the human monocyte-derived macrophages (HMDM) averaged 21.6 ± 3.0 mU/mg cell protein and did not differ among the three groups.

Conclusions. PreHTN subjects have higher ACE2-II activity compared with HTN subjects, suggesting a protective role for ACE2 in the early stage of HTN development, probably by accelerated degradation of the vasopressor AngII.

Keywords: ACE2; angiotensin; hypertension; macrophages; RAAS

Introduction

The renin–angiotensin–aldosterone system (RAAS) is considered to play an important role in maintaining a cardiovascular physiological homeostasis [1]. The RAAS axis initiates with renin cleavage of angiotensinogen to form the inactive decapeptide angiotensin I (AngI). The latter is subsequently converted by angiotensin-converting enzyme (ACE), a dipeptidyl carboxypeptidase, to the central peptide effector of the RAAS, angiotensin II (AngII). The biological activities of AngII include vascular constriction and enhancement of sodium retention in the kidney by stimulation of aldosterone secretion, which in turn can induce hypertension. By degrading the vasodilator peptide bradykinin, ACE further contributes to hypertension development.

A relatively new player in the RAAS is an ACE homologue, ACE2 [2,3]. ACE2 maps to a defined quantitative trait loci (QTL) on the X chromosome of rat models of hypertension and their mRNA and protein expression were markedly reduced in these hypertensive animals [4]. ACE2 enzymatic activities include the degradation of both AngI and AngII with the subsequent formation of Ang-(1–7) which is known to have vasodilatory action and other biological effects opposite to those of AngII [5,6]. Ang-(1–7) is formed either directly by ACE2-mediated hydrolysis of AngII or indirectly by ACE2-mediated hydrolysis of AngI to form Ang-(1–9), a peptide with unknown biological activity, followed by conversion to Ang-(1–7) by ACE.

Playing opposing roles in angiotensin metabolism, the balance of ACE and ACE2 activities may determine blood pressure (BP) homeostasis. The hypothesis explicitly formulated by Yagil and Yagil [7] assumes that ACE2 relative expression may have an important role in shifting the balance between...
AngII-induced vasoconstriction/hypertension and Ang-(1–7)-induced vasodilator/hypotension effects.

To test this hypothesis, we measured ACE and ACE2 activities in human monocyte-derived macrophages (HMDM) obtained from normotensive and untreated hypertensive subjects. HMDM, expressing both ACE and ACE2 activities [8], serve as a model cellular system. The present study focuses on a male cohort.

Methods

Patients and study protocol

A total of 57 male subjects were enrolled in the study. All participants were non-smokers with no known chronic disease and taking no medications. None of the participants was obese [body mass index (BMI) 22–26]. Three separate seated cuff BP measurements were taken by trained physicians using a mercury sphygmomanometer. A single 20ml blood sample in the fasting state was collected from each patient upon enrollment.

All patients signed an informed consent form and the study was approved by the Helsinki Committee of the Rambam Medical Center, Israel Ministry of Health.

Human monocyte-derived macrophages (HMDM) isolation

Anti-coagulated (heparin 10 U/ml) blood from fasting participants was layered over Histopaque (Sigma). After centrifugation, the mononuclear cell layer was collected, cells were washed and plated at 10^6 cells/ml (Primaria Brand, Falcon Labware) in RPMI medium, supplemented with 10% FCS (Biological Industries, Israel). After 2h adherence, the medium was replaced with RPMI supplemented with 10% autologous serum and antibiotics. Cultures were kept at 37° C in a humidified incubator (5% CO2, 95% air). The medium was changed every 48–72 h and HMDM cultures were tested following 5–7 days in culture.

Macrophage ACE activity determination

HMDM plated into 96-well plates were analysed for their ACE activity using a commercial kit (Sigma). The kinetics of ACE-mediated cleavage of the synthetic substrate furyl-acryloyl-phenylalanyl-glycyl-glycine to furyl-acryloyl-phenylalanine and glycine was measured by reduced absorbance at 340 nm [9]. The absorbance kinetic was measured in a UV microplate reader (PowerWave, Biotech) and standardized to a known calibrator activity. The activity for each subject represents the average (VAR < 10%) of a triplicate. Inter-assay variability was <10%.

ACE2 activity assay

ACE2 activity determination in HMDM is based on the method described by Huang et al. [10] and modified to measure cellular ACE2 activity [8]. Briefly, ACE2-I and ACE2-II activities are the respective hydrolysis of Ang I and Ang II, resulting in cleavage of leucine or phenylalanine at the C-terminal. The assay is based on measurement of free amino acid released upon addition of either Ang I or Ang II (10 nmol). With the addition of the respective dehydrogenase (0.5 U/ml) in the presence of β-nicotinamide adenine dinucleotide (NAD; Sigma), NADH is formed and the latter is coupled to diaphorase-mediated conversion of resazurine to resorufin which is fluorescent (excitation 565 nm, emission 585 nm). Fluorescence kinetics is measured for 1 h at room temperature in the Fluostar Galaxy plate reader (BMG Labtechnologies, Germany). Specificity was confirmed using a specific antibody directed against the ectopic domain of human ACE2 (R&D systems, Minneapolis, MN, USA).

Activity results are expressed as fmole leucine or phenylalanine formation per minute and normalized to milligram tissue protein. The activity for each subject represents the average (VAR < 10%) of a quadruplicate. Inter-assay variability was <10%.

Statistical analysis

Results of enzymatic activities are expressed as mean±SEM. One-way analysis of variance (ANOVA) followed by the Dunnett test was used for comparison of activities between groups. P < 0.05 is considered statistically significant.

Results

The subjects enrolled were 32.8±1.6 years of age. Three groups were classified according to the seventh report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC7) on high BP [11]: normotensive (NT; n = 28), prehypertensive (preHTN; n = 18) and hypertensive (HTN; n = 11) with seated systolic and diastolic BP averaging 112.6±1.2/78.1±1.4 and 151.4±2.7/99.3±2.4 mmHg, respectively (P < 0.001).

ACE2-mediated AngII hydrolysis (ACE2-II), measured in HMDM obtained from the study subjects, is demonstrated in Figure 1A. Average ACE2-II activity in NT subjects was 1201±241 fmol/min/mg cell protein. ACE2-II activity in HTN (1621±272 fmol/min/mg cell protein) was not significantly different from NT (P > 0.05). However, a 2.4-fold increase was measured in preHTN (2904±578 fmol/min/mg cell protein) compared with control NT subjects (P < 0.01).

AngII hydrolysis rate was significantly higher than AngI degrading activity. ACE2-II to ACE2-I activity ratio averaged ×85 and it did not differ significantly between the study categories.

No correlation was found between BP and ACE activity (Figure 1B). Average ACE activity was 21.6±3.0 mU/mg cell protein.

Discussion

This work demonstrates for the first time that ACE2-mediated AngII hydrolysis in HMDM obtained from...
Increased ACE2 in preHTN

It was previously reported [4] that under basal conditions, although expression of ACE2 in hypertensive-prone rats is lower than that in resistant strains, the differences in BP are mild. However, in response to hypertensive stimulus, resistant strains maintained normal BP while a significantly increased BP concomitant with a further reduced ACE2 was observed in HTN-prone rats. Therefore, it is suggested that HTN and preHTN subjects are exposed to hypertensive stimulus and the resulting level of BP could be determined by the expression of ACE2.

Increased ACE2 in preHTN compared with HTN was recently reported in spontaneously hypertensive rats [19], where ACE2 expression and activity are significantly increased at birth and dramatically fall with onset of HTN. Elevated ACE2 was also observed only in early stages of diabetes, and this rise disappeared with progression of the disease, suggesting a renoprotective role for ACE2 [20]. Further studies are required to determine whether an initial increased ACE2 activity is followed by a return to normal levels with the progression of preHTN to evident HTN. The biphasic regulation of ACE2, stimulation and shutdown, with the progression of pathophysiological conditions needs to be elucidated. It is suggested that maintaining elevated levels of ACE2 could decelerate the progression toward evident hypertension. In the Trial of Preventing Hypertension (TROPHY) Study [13], treatment of preHTN subjects with candesartan reduced the risk of incident hypertension. As antagonists of the AngII type I receptor were previously shown to increase ACE2 [21], it is suggested that some of the beneficial effect of candesartan could be attributed to this indirect effect.

The in situ catalytic efficiency for degrading AngII was previously shown to be about 400-fold higher than AngI [22]. In this work, we also demonstrate a significant preference to hydrolysis of AngII, which is about 85-fold higher than that measured with AngI in HMDM. The activity pattern of ACE2 observed in HMDM could indicate its possible role in the control of BP via an efficient removal of the vasopressor along with increased formation of the vasodilator. The alternative path ‘bypassing’ the formation of angiotensin II has probably only marginal importance. Since ACE activity did not differ significantly between BP categories, the observed changes in ACE2 activity would shift the angiotensin balance toward increased vasodilatory peptide level. However, the extent to which ACE and ACE2 relative activity contribute to the balance of angiotensins would be difficult to evaluate, since ACE2 activities were measured against the enzyme’s natural substrates and ACE activity was measured with a synthetic substrate. Compared with the present study, a higher ACE2-I activity was measured in our previous [8] study. The discrepancy of the results between the two studies could be explained by characteristics of the previous study population, severe heart failure as well as drug treatment, both previously shown to increase ACE2 [21,23]. As ACE2-II activity was not measured in the previous study,
we cannot exclude a possible change in the ACE2 substrate preference. In cardiac tissue of mice, we observed a significantly increased ACE2-I activity compared with ACE2-II following treatment with mineralocorticoid receptor blocker (unpublished data). Further studies are necessary to reveal the mechanism underlying the regulation of ACE2 level and substrate preferences in the cellular settings.

Although with a limited group size, the results presented in this report are highly significant. However, since BP control depends on concerted contribution of multiple cells, hormones and environmental factors, the results of our study must be interpreted cautiously, especially in light of its limitation to only one cell type, HMDM. A growing body of evidence identifies tissue RAAS [24,25] as an important factor in the development of cardiovascular disease, independent of circulating RAAS. Macrophages—tissue resident cells—were employed in this study as a model system to examine tissue expression of ACEs. We have recently shown that modulation of ACE2 activity in mouse macrophages resembled changes in their heart activity [8] and it was recently shown [26] that enzymatic activities are tissue-specific, sensitive to hypertension programming. Similarly, ACE2 activity measured in HMDM with respect to BP could either express a direct contribution of macrophages to the local RAAS [27] or reflect the pattern of activity in the heart or arterial wall, thus having a direct relevance to control of BP. Alternatively, as macrophage infiltration and activation [27] play a key role in the development of HTN target organ damage, where both elevated BP and increased AngII are known risk factors, the increased expression of ACE2 in these cells may attenuate the progression of the disease.

To summarize, our results suggest that significantly elevated ACE2 activity in preHTN subjects might have a protecting effect in the development of hypertension, through the ability of this enzyme to counteract the AngII vasoconstrictive effect. However, in HTN subjects, ACE2 activity would probably mean the failure or ‘insufficient’ ability to provide such protection.

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Conflict of interest statement. None declared.

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