Angiotensin-converting enzyme and angiotensin II receptor 1 polymorphism in coronary disease and malignant ventricular arrhythmias

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Abstract

Objectives: It has been reported that patients carrying the angiotensin-converting enzyme (ACE) deletion DD genotype with the angiotensin II type 1 (AT1) C allele are at increased risk for myocardial infarction. The frequency distribution of the ACE and AT1 receptor gene polymorphism and their possible relation regarding malignant ventricular arrhythmias in patients with coronary artery disease (CAD) and left ventricular dysfunction was determined. Methods: The ACE I/D and AT1 A/C polymorphisms (using polymerase chain reaction) in 100 Caucasian patients suffering from CAD with a history of malignant ventricular arrhythmias treated with an implantable cardioverter defibrillator (ICD group) was compared to 127 age-matched Caucasian patients with CAD and no history of malignant ventricular arrhythmias (control group). All patients had reduced left ventricular ejection fraction of <40% and were comparable regarding sex distribution, body mass index, ACE-inhibitor treatment, lipid status and duration of CAD. Results: The prevalence of DD/CC in the ICD group was significantly higher (19% versus 10%, p = 0.0001). The risk for malignant ventricular arrhythmias was associated with the combination of ACE D and AT1 C alleles (odds-ratio: 2.4, 95% confidence interval 1.41 to 3.94, p = 0.001). The distribution of ACE and AT1 genotypes was not different between the two groups. Conclusions: Patients with coronary artery disease and left ventricular dysfunction carrying ACE D and AT1 C alleles are at increased risk for development of malignant ventricular arrhythmias. Because of available pharmacological inhibitors, these results may have clinical implications for the prevention of sudden cardiac death. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

The angiotensin-converting enzyme (ACE) is an ectoenzyme mostly located in endothelial cells and plays a central role in the regulation of the renin–angiotensin system (RAS) with several physiological properties: regulation of circulating angiotensin II (AT II); increase of bradykinin breakdown; interference with cardiac tissue AT II formation [1–3].

In humans an insertion/deletion polymorphism in the gene encoding ACE results in genotypes II, ID and DD [4]. The DD genotype has been shown to result in high plasma concentration of ACE. The frequency of the ACE DD genotype is increased in the presence of ventricular hypertrophy [5], dilated cardiomyopathy [6] and in patients with hypertrophic cardiomyopathy with a strong history of sudden cardiac death (SCD) compared with unaffected relatives [7].

The angiotensin II type 1 (AT1) receptor, which is located in vascular smooth muscle cells and myocardium, mediates most of the actions of angiotensin II. A polymorphism in the 3' untranslated region of the gene encoding human (AT1) receptor corresponding to an A/C
transversion has been identified [8]. Tiret et al. [9] found a significant interaction between the ACE II/DD polymorphism and the AT1 A/C gene polymorphism: the risk for myocardial infarction was higher in homozygotes subjects with a DD/CC constellation. These results have been confirmed in association with early coronary artery disease (CAD) in patients aged 50 years or less [10].

Clinical trials have shown that in patients with congestive heart failure, treatment with ACE inhibitors is associated with a reduced mortality from SCD caused by malignant ventricular arrhythmias [11–13]. Regarding the physiological role of the RAS, the polymorphisms of both ACE and AT1 receptor gene as well as their potential interaction could be involved in the pathogenesis of the malignant ventricular arrhythmias.

In this study we investigated this hypothesis in patients with coronary artery disease (CAD) and left ventricular dysfunction with a history of malignant ventricular arrhythmias who subsequently were treated with an implantable cardioverter defibrillator (ICD) compared to those without a history of malignant ventricular arrhythmias.

2. Methods

2.1. Study population

A total of 227 homogenous Caucasian patients from the same region (Vienna, lower Austria, Upper Austria; Austria) were recruited. All patients had reduced left ventricular ejection fraction (LVEF<40%) determined by radionuclide ventriculography. One hundred consecutive patients with CAD were survivors of SCD with documented ventricular tachycardia and/or fibrillation not associated with acute myocardial infarction. These patients were subsequently treated with an ICD (ICD group). The control group consisted of 127 age-matched patients suffering from CAD and no history of malignant ventricular arrhythmias. Informed consent was obtained from all patients, following the recommendation of the World Medical Association Declaration of Helsinki [14].

2.2. ACE I/D polymorphism

ACE polymorphism was detected as previously described [15]. Genomic DNA from leukocytes are prepared by standard techniques. The primers used to encompass the polymorphic region of the ACE were: 5′-CTGGAGAC-CACTCCCATGTTTA-3′ and 5′-GATGTTGGCACATCCATTGCAGAT-3′. The polymerase chain reaction (PCR) contains 1 μg of DNA template, 140 nmol of each primer, 200 μmol of four dNTPs, 2.5 units of Taq DNA polymerase and 3.0 mmol MgCl2. DNA is amplified for 30 cycles (initial melting at 95°C for 1 min, denaturation at 94°C for 1 min, annealing at 58°C for 1 min, extension at 72°C for 1 min) with a final extension time of 7 min. The PCR products are separated on 2% agarose gel and identified by ethidium-bromide staining. Furthermore, we used an insertion-specific amplification to avoid DD mistyping. All samples found to be DD were re-amplified with insertion-specific primer pair which recognizes the inserted sequence: 5′-TGGGACACAGGCCC-GCCCCACTAC-3′ and 5′-TCGCCAGCCCTCCCAT-GCCCATTA-3′. A 100-μl mixture of 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris–HCl, 250 mM dNTPs, 0.4 mM primers.

2.3. AT1 A/C polymorphism

The primers used to amplify the AT1 receptor gene were: 5′-ATAATGTAAGCTCATCACCAGAAG-3′ and 5′-CTCGCTTTACATTGAAAAGTACTTAA-3′. The hybridization procedure was slightly modified [16]: The ACTA1 primer was end-labeled with (32 P) dATP using T4 polynucleotide kinase. Amplification by PCR was carried out in a total volume of 25 μl using 50–200 ng of genomic DNA, 50 mM KCl, 0.01% gelatin, 5 mM Tris–HCl (PH 8.3), 1.5 mM MgCl2, 100 μM deoxynucleotides, 1.5 units of Taq polymerase (Boehringer Mannheim) and 10 pmol of each primer. A thermal cycler was used for 30 s at 95°C and 20 s at 56°C. The initial denaturation stage was carried out at 95°C for 4 min. The length of each allele was determined on a 6% denaturing agarose gel.

2.4. Radionuclide ventriculography

Left ventricular function were assessed quantitatively by gated blood pool radionuclide ventriculography as described before [17]. Values were calculated using a standard variable region-of-interest technique. LVEF values <50% were considered to be abnormal.

2.5. Statistical analysis

Statistical analyses were performed with SAS software, version 6.08. Data are expressed as mean and SD. Statistical significance was defined as p<0.05.

Quantitative parameter were compared with analysis of variance (ANOVA) according to the two study groups.Categorical characteristics were compared by chi-square test. The distribution of ACE gene and AT1 polymorphism were also compared by chi-square test. Adjusted odds ratios were computed from linear logistic regression models for the binary response data (ICD and controls) and ordinal response (II, ID, DD; AA, AC, CC), respectively by the method of maximum likelihood. The linear logistic model has the form: g (p) = α + βx; where g is the link function, α the intercept parameter and β is the vector of the slope parameters.
Table 1
Baseline characteristics

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>ICD</th>
<th>Control</th>
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<td>100</td>
<td>127</td>
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| Age, years      | 63±9 | 62±10   |
| Males, %        | 83   | 78      |
| Body mass index, kg/m² | 26.4± 4.6 | 27.5± 3.9 |
| Total cholesterol, mg/dl | 208±43 | 205±45 |
| HDL, mg/dl      | 46±12| 44±11   |
| LDL, mg/dl      | 135±54| 137±51 |
| Triglycerides, mg/dl | 171±94 | 171±89 |
| Duration of CAD, years | 10±7  | 8±7     |
| Previous MI, %  | 88   | 74      |
| ACE-inhibitor, % | 68   | 57      |
| EF, %           | 28±13| 25±7    |

ACE: angiotensin converting enzyme; CAD: coronary artery disease; EF: left ventricular ejection fraction; HDL: high density lipoprotein; LDL: low density lipoprotein; ICD: implantable cardioverter defibrillator; MI: myocardial infarction.

3. Results

The demographic characteristics of the overall sample are summarized in Table 1. The study groups were comparable with regard to sex distribution, body mass index, ACE treatment, lipid status and duration of CAD.

3.1. ACE gene polymorphism

The frequency distribution of the ACE receptor polymorphism is summarized in Table 2. The frequencies of I/D in the ICD group was 31% for DD, 49% for ID and 20% for II. In the control group 40% of the patients had DD, 41% ID and 19% had II genotypes. There was no significant difference in the distribution of the I and D allele between the ICD group and the controls.

3.2. AT1 polymorphism

Table 2 shows the genotype and allele frequencies of the AT1 polymorphism. In the ICD group 32% of the patients had AA, 38% had AC and 30% CC genotype. In the control group the frequency distribution of AT1 receptor gene was 43% for AA, 39% for A/C and 17% for CC. The prevalence of the CC homozygotes was higher in the ICD group compared with the controls. This difference, however, was statistically not significant (p = 0.054).

3.3. ACE and AT1 polymorphism

The frequency distribution of the ACE polymorphism according to the AT1 genotype is shown in Table 3. In the ICD group 31 patients with DD genotype 23% had also a CC genotype. In the control group the prevalence of CC was 15% out of 51 patients with DD genotype. This difference was statistically significant (p < 0.0001). There was an association between the combination of ACE D and AT1 C alleles on the risk of malignant ventricular arrhythmias. The risk for malignant arrhythmias approximated by the adjusted odds ratio was 2.4 per number of D/C alleles (95% confidence interval, 1.41 to 3.94 p < 0.001). Thus, individuals carrying DD/CC genotype were at highest risk.

4. Discussion

To the best of our knowledge, this is the first report investigating the association between ACE and AT1 polymorphism and malignant ventricular arrhythmias in patients with coronary artery disease and impaired left ventricular function. In this study, the prevalence of the DD genotype was comparable in both groups to that obtained by others in patients with CAD [9,18]. There was a nonsignificant increased prevalence of CC homozygotes in the ICD group, while the AT1 allele frequencies in the control group were in accordance to those previously reported in patients with cardiovascular disease [10,19]. However, the risk of malignant ventricular arrhythmias seems to be associated with the combination of ACE D and AT1 C allele and the subset of individuals carrying DD/CC alleles are highest risk.

Among multiple factors involved in the pathogenesis of sudden cardiac death, the RAS is of particular interest.
because of available pharmacological inhibitors. Possible arrhythmic mechanisms of the RAS:

1. ATII-induced stimulation of the Ca\(^{2+}\) channels is mediated by a Na\(^{+}\)–H\(^{+}\) antiport resulting in Ca\(^{2+}\) overload in cardiac myocytes [20]. This inotropic effect has a major clinical significance in the presence of impaired left ventricular function of ischemic etiology, and hence may explain an ATII facilitation of ventricular arrhythmias.

2. In experiments where the role of ATII on infarct size and reperfusion arrhythmias have been examined, reperfusion arrhythmias was reduced in AT1-receptor-deficient mice and AT1-blocker-treated mice [21]. ATII, therefore may be critically involved in the induction of ventricular arrhythmias.

3. ATII, by increasing afterload and left ventricular systolic stress, may play a role in the modulation of the electromechanical feedback. These conditions which produce abnormal stress i.e. increased chamber diameter, high intracavity pressure and changes in inotropic state are associated with anomalies of several electrophysiological variables such as dispersion of refractoriness, reduced action potential duration, induction of early and delayed afterdepolarisation [11].

4. Sympathetic influences are important determinants of ventricular arrhythmias. An increased sympathetic tone [22] and stimulation of RAS is found during congestive heart failure. ATII, in turn, enhances neurotransmitters release from presynaptic nerve endings and increases catecholamine biosynthesis [23].

5. Decreased parasympathetic activity accompanied by a reduced heart rate variability has been linked to poor prognosis in patients after myocardial infarction [24]. ATII provokes a central inhibition of cardiac parasympathetic tone probably by acting on structures supplied via the vertebral arteries in, or near to, the area postrema [25].

6. Cardiac myocytes, vascular smooth muscle cells, and fibroblasts possess ATII receptors and show a growth enhancing effect [26]. Therefore, cardiac remodeling can present potential arrhythmic substrate due to facilitation of anisotropic reentry [27].

Regarding the mechanisms mentioned above a variability of the activity of the RAS, because of genetic variation of the constituents, may therefore predispose patients with ischemic heart disease to malignant ventricular arrhythmias. Systemic variations were found to be partly the reason of an I/D polymorphism, which is the cause for the interindividual variability of ACE plasma levels [4]. The DD genotype deserves special attention because it is correlated with higher plasma ACE and therefore faster conversion of ATI to ATII [4].

In humans the effect of ATII seems primarily be mediated by AT1 receptors. Among several diallelic polymorphisms encoding the AT1 receptor gene one variant, an A/C transversion, was associated with cardiovascular phenotypes [8]. The allelic frequency of C was significantly higher in hypertensive patients.

We observed an association between ACE D and AT1 C alleles with respect to malignant ventricular arrhythmias. The biological mechanism of this interaction on risk of malignant arrhythmias is unknown. In twins without overt cardiac disease the ACE DD genotype has been associated with increased heart rate variability [28], which is thought to be protective against ventricular arrhythmias. In our study, however, the AT1 genotypes rather than ACE genotypes appear to be involved in the risk for malignant arrhythmias. This finding is in accordance with the fact that reduced heart variability in patients with ischemic cardiomyopathy is due to a central inhibition of cardiac parasympathetic tone induced by ATII [25].

In contrast to ACE D allele with enhanced expression of ACE, no functional diversity with the A/C transversion per se has been identified. Therefore, the AT1 C allele can be considered as a possible marker, in linkage disequilibrium with other genetic variants. Since in a rat model AT1 receptor blockade was associated with decreased plasma and tissue ACE activities [29], increased activity of the AT1 receptor, under genetic control, could be associated with over expression of the ACE gene. Moreover, studies from isolated human cardiac tissue conclusively show that, approximately 80%–90% of the ATI conversion in the ventricles is carried out by a serin protease isolated from the ventricle not inhibited by captopril [2]. ACE is responsible for approximately 10%–20% of the ATI conversion. Since AT1 receptors are present in myocardium, the presence of this alternate pathway may present a potential escape by which the gene variants could interact. The importance of this pathway is further confirmed by a study [30] demonstrating that in a population with congestive heart failure the treatment with losartan a non-peptide AT1 receptor antagonist reduced total mortality compared with an ACE inhibitor such as captopril. This mortality advantage for AT1 blocker appears primarily to be due to a reduction in SCD.

In a recent study Amant et al. [31] demonstrated that the AT1 CC genotype is associated with exaggerated vasoconstriction in distal coronary arteries. Although the biological relevance of the AT1 A/C polymorphism is unknown at the present time, the differences between CC subjects and other two genotypes is compatible with potential differences of clinical relevance. It is therefore, conceivable that a transient completely reversible ischemic episode, in the presence of extremely variable degrees of coronary sclerosis, can be involved in the genesis of the malignant ventricular arrhythmias. The AT1 CC genotype may favor or predispose the development of electric instability and SCD as a consequence of events resulting from enhanced coronary vasmotion. The presence of chronic structural abnormalities of the myocardium may further play an
adjunctive role for generation of fatal arrhythmias in the population with ischemic heart failure.

In conclusion these results, may have clinical implication for the prevention of sudden cardiac death in patients with coronary artery disease and left ventricular dysfunction. Regarding the potential relevance of these genes in the modulation of ventricular arrhythmias and available pharmacological ACE and AT1 inhibitors, further studies are needed to address this question in independent populations.

References