Original article

The pro-oxidant effect of angiotensin-1 converting enzyme in Tunisian patients with coronary heart disease

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Abstract. *Background:* Evaluation and investigation of the pro-oxidant role of the angiotensin-1 converting enzyme among Tunisian coronary. *Materials and methods:* In the present study the angiotensin-1 converting enzyme (ACE1) was determined by a kinetic method for coronary and witness populations. These subjects (117 patients and 86 controls) beneficed also by an enzymatic determination of superoxide dismutase (SOD), glutathione peroxidase (GPx) and total antioxidant status (TAS) to reveal the atherogenic effects of these radical species and investigate their interactions with ACE1. *Results:* The determination of ACE1 activity showed a significant increase in patients compared to controls (82.24±21.6 vs 49.23±12.85 UI/L, p<0.000001). Statistical tests have shown negative correlations between the ACE 1 activity and the antioxidant defense markers (SOD, GPx and TAS). *Conclusion:* In addition to its vasoconstrictor role, ACE1 can be considered as a pro-oxidant enzyme, these two effects combine in the genesis and the complications of cardiovascular diseases. (www.actabiomedica.it)

Key words: Angiotensine-1 converting enzyme, Acute Coronary Syndrome, Pro-oxidant effect, risk factor

Introduction

Acute coronary syndrome (ACS) is classified among the major causes of mortality and morbidity in industrialized countries (1-3).

Atherosclerosis is the principal etiology of this coronary disease. Arterial hypertension, diabetes, smoking, obesity and hypercholesterolemia are the classic risk factors of these multifactorial and polygenic diseases (4-6).

Angiotensin-1 Converting enzyme (ACE1) (peptidyl-dipeptidase A; EC,3,4,15,1) is well known by its physiological role in the renin angiotensin system by cleaving angiotensin 1 (an inactive decapeptide) to generate angiotensin 2 (a powerful vasoconstrictor) and hydrolyzed bradykinin (a potent vasodilatator). This enzyme stimulates also the release of aldosterone from the adrenal cortex, leading to sodium ion retention (7-9). Beside this functions, the ACE1 plays a pro-oxidant role in the umbra through the activation of NADPH oxidase responsible for the generation of superoxide ions (by angiotensin 2 neo activated)causing an imbalance in the prooxidant-antioxidant balance (8, 10, 11). An over expression of this enzyme (usually of genetic origin) favors the ACS's genesis and complications (5, 12, 13).

In this context, our study aims to evaluate the ACE1 activity in Tunisian coronary patients, andinvestigate its pro-oxidant effect on these patients by measuring the markers of the first line of defense against aggression radical, superoxide dismutase (SOD) and glutathione peroxidase (GPx) and also by quantifying the total antioxidant status (TAS).

Materials and methods

Study Population

This is a prospective study, in which sampling was carried between January and November 2010. One hundred seventeen Tunisian coronary subjects (86 men and 31 women) middle-aged (64.8±11.7 years) were recruited from the Cardiology Service of CHU Farhat Hachedof Sousse, Tunisia.

Eighty six healthy subjects (64 men and 22 women) middle-aged (54.8±9.4 years) represented the control group.

The patients and witness signed a free and clear consent which explains the objectives of this work with an undertaking not to publish the names of participants, their personal data including test results. Andatasheet had been prepared for each subject (patient or control) to identify cardiovascular risk factors and to know the susceptibility degrees to ACS. This sheet contains the anthropometric characteristics,the biological parameters, the risk factors, the treatments of patients, the exclusion factors.

Laboratory Analysis

Venous blood samples were drawn after 12 hours overnight fast, a prelevement of three tubes were made for each patient and witness: a tube without anticoagulant for determination lipid parameters, total antioxidant status (TAS), and ACE1 activity and, an heparinized tube for glucose, glutathione peroxidase (GPx) and superoxide dismutase (SOD) and a third EDTA tube to determine hemoglobin.

Serum total cholesterol (TC), triglycerides, high density lipoprotein cholesterol (HDL-C) and glucose were measured with colorimetric essay using an automated system (Cx9 Pro-Bechman Coulter-Fuller-Ton CA). Low density lipoprotein cholesterol (LDL-C) was determined by Friedewald formula for TG levels below 4.5 mmol/L

Apolipoproteins (ApoliporoteinA1, Apolipoprotein B, lipoprotein Lp(a) were determined by immunenephelometry (Dade Behring, Marburg, Germany) and ACE1 activity by kinetic method at 340 nm using a synthetic substrate (Furylacryloyl-phenylalanyl – glycyl-glycine(FAPGG)) (Trinity Biotech, St Louis USA).

Erythrocyte SOD activity was determined by colorimetric enzymatic method to xanthine oxidase at 510 nm (Randox, Antrim, UK) and GPx activity by enzymatic method at 340 nm (Randox, Antrim, UK). The TAS was measured by colorimetric method at 600 nm (Randox, Antrim, UK), and the hemoglobin rate by the Drabkin method.

Statistical Analysis

Database management and statistical analyses were carried out using SPSS (Statistical Package for the Sociological Sciences), version 13.0. Results are presented as means ± SD, or percentages. Means were compared using Student test. The relations between variables were assessed with Pearson's correlation analysis. The significance threshold was set at 5% ($p<0.05$).

Results

Table 1 summarizes the anthropometric and clinical data of patients and controls. Hypertension, diabetes and tobacco are the major risk factors;by against our control population is not exposed to these factors. Table 2 illustrates the variations of glucose, lipid markers, apolipoproteins, the SOD erythrocytes activity, GPx erythrocytes activity, total antioxidant status (TAS) and ACE1 activity. Glucose, apolipoprotein B (Apo B) and lipoprotein Lp (a) concentrations were significantly elevated in patients compared tocontrols unlike apolopoprtéine A1 concentration was significantly higher among controls compared topatients.Lipid markers (TC, HDL-C, LDL-C and TG) showed no significant difference between patients and controls.

In our study, a statistically significant elevation of the first-line antioxidant parameters (SOD and GPx) was found among patients compared with controls. Contrary, total antioxidant status (TAS) was statistically elevated in controls compared to patients.

For the ACE1 activity, although the majority of our patients (92%) were treated with ACE1 in-

Table 1. Anthropometric characteristics and clinical data of patients and controls

	Patients (n=117)	Controls (n=86)
Age (x±σ years)	64.8±11.7	54.8± 9.4
Sex		
Men (%)	73.5	74.5
Women (%)	26.5	25.5
BMI	27.8±4.6	24.2±2
Hypertension (%)	61.5	0
Obesity (%)	40	0
Diabetes (%)	52	0
Smoking (%)	50	0
Family cardiac history (%)	37	0
Personnel cardiac history (%)	35	0
postmenopausal women (%)	100	63
Dyslipidemia (%)	32.5	0
Sedentary (%)	17	0
Alcohol (%)	32.5	19
Treatment:		
ACE1 inhibitors (%)	92	0
Statins (%)	29	0
Beta-Blockers (%)	34	0
Ca-Blockers (%)	22	0
Diuretics (%)	36	0

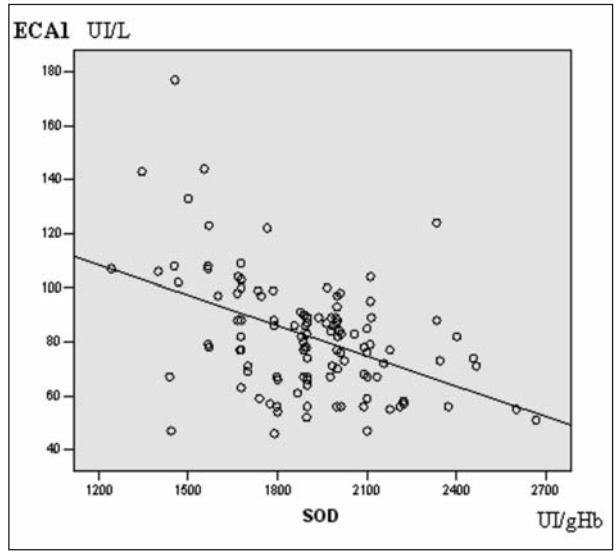

Figure 1. The correlation between ACE1 and SOD activity in patients population

hibitors, this activity was statistically elevated in patients compared to controls.

The investigations of the relationship between angiotensin-1 converting enzyme and the oxidative stress parameters showed a statistically significant negative correlation between this activity and SOD values (r=-0.42, p<0.0001) (Figure 1), a significant negative correlation was also established between the ACE1 activity and GPx values (r=-0.64, p<0.0001) (Figure 2). The interactions between the two antioxidant markers GPx and SOD were established in Figure 3 and a significant positive correlation (r=0.41, p <0.0001 was drawn.The metabolic interactions between ACE1 activityand the TAS values were illustrated in Figure 4,a statistically negative correlation (r =-0.34,p <0.0001) was established between the two markers.

Table 2. The distribution of apolipoproteins, ACE1 activity and anti-oxidant markers in patients and controls subjects

Populations Biological parameters	Patients (n=117)	Controls (n=86)	p
Glucose (x±σ mmol/L)	9.22±5.23	5.10±1.42	<0.0001
HDL-C (x±σ mmol/L)	1.41±0.33	1.45±0.31	NS
LDL-C (x±σ mmol/L)	2.75±0.44	2.77±0.62	NS
TC (x±σ mmol/L)	4.7±1.14	4.6±0.86	NS
TG (x±σ mmol/L)	1.75 ± 0.60	1.55±0.32	NS
ApoA1 (x±σ, g/L)	1.03±0.2	2,02±0,41	<0.0001
ApoB (x±σ, g/L)	1.9±0.4	0.96±0.42	<0.0001
Lp (a) (x±σ, g/L)	0.37±0.1	0.21±0.024	<0.0001
ACE1 (x±σ, UI/L)	82.24±21.6	49.23±12.85	<0.000001
GPx (x±σ, UI/gHb)	63.38±10.9	46.29±8.7	<0.000001
SOD (x±σ, UI/g/Hb)	1901±341	1488±281	<0.000001
TAS (x±σ, mmol/L)	1.88±0.018	2.15±0.019	<0.0001

NS: not significant

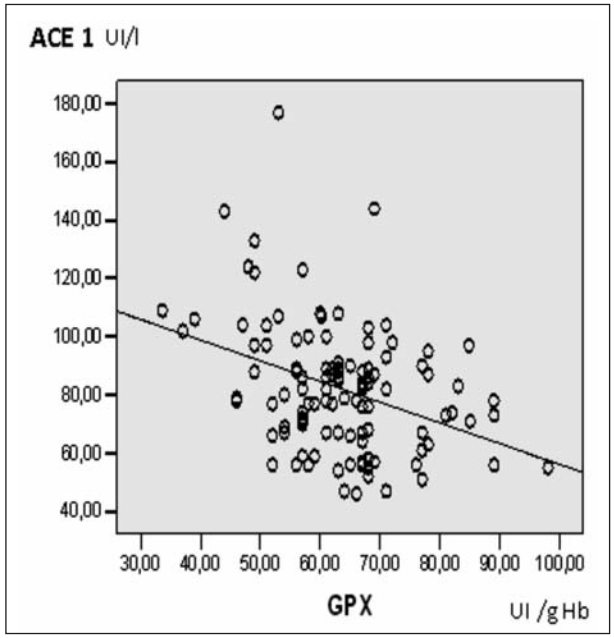

Figure 2. The correlation between ACE 1 and GPx activity in patients population

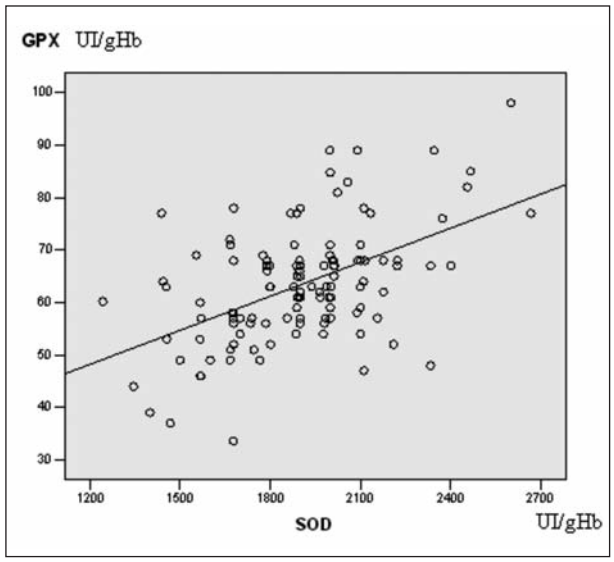

Figure 3. The correlation betweenGPx and SOD activity in patients population

Discussion

Varied risk factors among patients confirm the multifactorial origin of the ACS, well explained by the Framingham study (5, 14). The patients lipid profile does not present any trouble thanks to lipid-lowering treatment and recommended diet

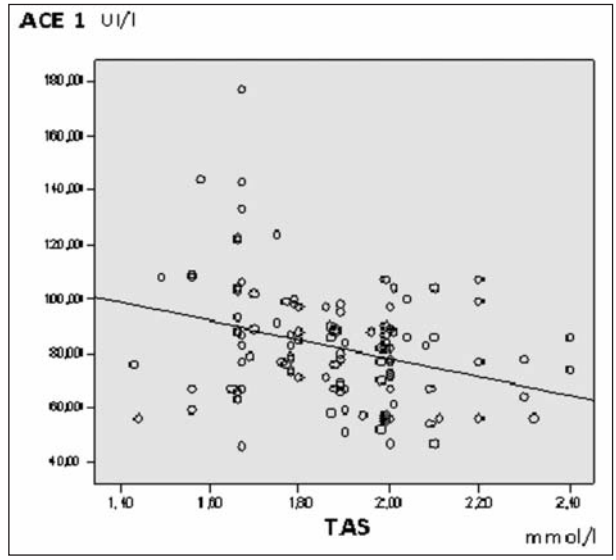

Figure 4. The correlation between ACE 1 activity and TAS concentration in patients population

Increased concentrations of apolipoprotein B and apolipoprotein Lp (a) consolidate their atherogenic effects and their involvement in the genesis of ACS, whereas Apo A1 (statistically higher in controls) has a cardioprotective effect supported by various studies (15-16).These apolipoproteins are under genetic control which showed the polygenic origin of ACS (15, 16).

Although, 92% of patients are under treatment with inhibitors of angiotensin-1 converting enzyme, this enzyme's activity was statistically higher among these patients. Reflecting the involvement of ACE1 as a riskparameter for heart diseases, this role is well described in the literature and explained mainly by hypertensive and vasoconstrictor effects of this zinc-metallopeptidase (5, 8, 17). Recent studies also began the study of pro-oxidant role of this enzyme by activation of NADPH oxidase which triggers lipid peroxidation starting point for atherosclerosis. Actually, the two vasoconstrictor and pro-oxidant effects accumulate in the pathophysiology of atherosclerosis and acute coronary syndrome (10, 18, 19).

The study of the antioxidant parameters of the first line of antioxidant defense (erythrocyte activity of

SOD and GPx) showed a significant increase in its parameters among patients compared tocontrols. This could be consistent with several studies particularly the Bartoszstudy (20, 21). Hyperactivity of these enzymes may reflect a kind of adaptive response towards a state of imbalance pro-oxidant antioxidant; these enzymes must act to reduce the amplification rate of radical species. Some studies explain the rise in the activity of these enzymes by a genetic regulation mediated by redox sensitive transcription factors such as Nrf2 (nuclear factor-erythroid-2 p45-related factor) (20, 22).

Although the measure of total antioxidant status (TAS) quantifies the cumulative action of antioxidants in the human body,this status was significantly decreased in patients compared to controls which brings us back to promote the role of other antioxidants (enzymatic and nonenzymatic antioxidants) and confirms theoxidative imbalance theory, this is a deficiency in the total antioxidant activity in patients (23, 24).

Statistically positive correlation between SOD and GPx is expected and proven by the antioxidant cascade during the neutralization of oxygen radical species (19, 22).

The negative correlations between the angiotensin-1 converting enzyme activity and various markers of oxidative stress (SOD, GPx and TAS) can be explained by the pro-oxidant effect of the ACE1, since this enzyme is responsible for the production of NADPH oxidase, an overactive SOD and GPx to neutralize the radical species generated (18, 19, 25-27).

Two hypothesis are suggested to explain these relationships; the first one is that gene regulation at the level of transcription factors would inhibit gene expression of ACE1 after activating the genes of the first line antioxidant (SOD, GPx ...). The Nrf2 pathway is the most detailed in the literature. This factor stimulated by radical species and oxidized metabolites activates the antioxidant defense by overexpression of enzymatic antioxidants and detoxificationof pro-oxidant metabolites (28, 29).The second hypothesis suggests the role of radical oxygen species generated following the activation of NADPH oxidase in the destabilization and damage to the endothelium principal secretory of ACE1 which leads to the diminution of its secretion and therefore its activity, to other hand, the total antioxidant status(TAS) was exhausted by the various toxic and pro-oxidant effects triggered during the oxidative imbalance (30, 31).

Conclusion

Our retrospective study is in agreement with several literature results. The multifactorial origin remains the common denominator of all studies of coronary heart disease since the Framingham cohorts.This study show the involvement of an ACE 1 in coronary syndromes, not only by its vasoconstrictor effect but also by its pro-oxidant effects implicated in the genesis and complication of coronary syndromes.

Oxidative imbalance is not only generated by the hyperactivity of the angiotensin-1 converting enzyme,the literature data incriminate other risk factors (hypertension, diabetes ...) in this imbalance (31-33).

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