Annexin V Levels in Survivors of Early Myocardial Infarction
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Introduction and objectives. Annexin V has an anticoagulant effect in vitro that derives from its ability to displace coagulation proteins from phospholipid surfaces, prolonging phospholipid-dependent coagulation reactions. Antiphospholipid antibodies (APL) and annexin V have an affinity for anionic phospholipids, so it has been hypothesized that one of the thrombotic mechanisms of APL may be due to displacement of annexin V from phospholipid surfaces.

We studied plasma annexin V levels and analyzed its relationship to risk factors and several blood markers.

Patients and method. We studied 62 patients < 45 years old who had suffered myocardial infarction. The control group comprised 23 healthy subjects of similar age and sex. We analyzed the presence of APL, anti-β2 glycoprotein I (β2-GPI), anti-β2-GPI/phospholipid complexes and anti-annexin V antibodies.

We determined plasma annexin V levels. Cholesterol, HDL-cholesterol, triglycerides, anti-annexin V antibodies. We did not detect any positive APL or anti-β2-GPI antibodies. In the control group there was only 1 patient with positive APL and anti-β2-GPI antibodies. The myocardial infarction group showed significantly lower levels of annexin V than the control group: 0.640 ng/ml (0.520-0.818 ng/ml) vs 1.570 ng/ml (1.140-2.390 ng/ml), p < 0.01. There were no statistical associations between annexin V levels and other variables.

Conclusions. The low levels of annexin V in young myocardial infarction patients could indicate a procoagulant trend. This hypercoagulable state was unrelated to the presence of APL.

Key words: Myocardial infarction. Thrombosis. Antibodies.

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Anexina V en pacientes supervivientes de un infarto de miocardio prematuro

Introducción. La anexina V presenta un importante efecto anticoagulante in vitro debido a su habilidad para desplazar las proteínas de la coagulación de la superficie de los fosfolípidos. Se cree que uno de los mecanismos patogénicos de los anticuerpos antifosfolípidos (AAF) podría ser debido a un desplazamiento de la anexina V de la superficie de los fosfolípidos de membrana. Hemos estudiado la concentración de anexina V analizando su relación con los factores de riesgo cardiovascular y diferentes marcadores hematológicos.

Pacientes y método. Se estudiaron 62 pacientes que habían sufrido un infarto de miocardio prematuro y 23 sujetos control. Se determinó la concentración plasmática de anexina V y la presencia de los siguientes anticuerpos: AAF, anti-β2 glucoproteína I (β2-GPI), anti-β2-GPI/unidos a fosfolípidos y anti-anexina V. Se determinaron los valores de colesterol, colesterol HDL, triglicéridos, activador tisular del plasminógeno y su inhibidor, von Willebrand factor, y fibrinógeno levels were measured.

Resultados. Se detectó sólo 2 pacientes con positivo anti-β2-GPI/phospholipid complexes and 2 pacientes con positivo anti-annexin V antibodies. We did not detect any positive APL or anti-β2-GPI antibodies. In the control group there was only 1 patient with positive APL and anti-β2-GPI antibodies. The myocardial infarction group showed significantly lower levels of annexin V than the control group: 0.640 ng/ml (0.520-0.818 ng/ml) vs 1.570 ng/ml (1.140-2.390 ng/ml), p < 0.01. There were no statistical associations between annexin V levels and other variables.

Conclusiones. La menor concentración de anexina V en jóvenes pacientes de infarto de miocardio podría indicar una tendencia protrombótica. Su papel protrombótico no parece estar relacionado con la presencia de AAF.

Palabras clave: Infarto de miocardio. Trombosis. Anticuerpos.
INTRODUCTION

The event of myocardial infarct (MI) is the result of a combination of environmental factors and the individual predisposition of each patient. Those patients who have a MI at an early age have been exposed during a brief time to cardiovascular risk factors and show, in addition, minor arteriosclerotic signs on angiographic study. For this reason, the role of prothrombotic factors may be of greater importance in this population. The study of these factors in early MI might allow for understanding the possible implications of hypercoagulability in the pathogenesis of acute coronary syndromes.

The elevation of certain hemostatic factors appears to play a vital role in the development of coronary diseases. Various prothrombotic factors and markers of endothelial damage have been associated with an increase in the risk of myocardial infarct, among them fibrinogen, tissue plasminogen activator (t-PA), and the von Willebrand factor.

Annexin V (ANV) is a calcium-dependent glycoprotein with a potent anticoagulant capacity in vitro (mainly as results of its negatively charged membrane phospholipids), inhibiting the prothrombinase and X-asa complexes and reducing plaque adhesion and aggregation. Circulating ANV can be released from the cells of the vascular wall (endothelial cells, smooth muscle cells) or from secretor cells of the spleen and liver; once it is in the plasma, it binds to blood cells (platelets and erythrocytes) or to endothelial cells. ANV appears to form an «antithrombotic shield» around the phospholipids, displacing their coagulation factors, and capable of inhibiting the prothrombinase and X-asa complexes, and reducing plaque adhesion and aggregation. In addition, ANV possesses high apoptotic cell affinity, since these cells produce a large amount of phospholipids, particularly phosphatydilserine.

On the other hand, it has been proposed that ANV could play a fundamental role in the thrombogenic mechanisms of the antiphospholipid antibodies (APA). IgG fractions in patients with APA reduce the presence of ANV in trophoblastic and endothelial cell cultures, producing an increase in the amount of anionic phospholipids capable of initiating coagulation. It is known that the presence of APA has been associated with a state of hypercoagulability and, although the appearance of MI as a manifestation of the antiphospholipid syndrome does not occur frequently, it has been proposed that the presence of APA in all young patients who have an MI should be systematically studied. Nevertheless, the true importance of the APA in MI is controversial.

Recently, our group has shown how a Cytosine to Thymine transition, in the Kozak sequence of the gene that codifies ANV, is an independent protective factor for the development of a premature myocardial infarct. Such a polymorphism permits greater efficacy in protein translation, and higher ANV values in the plasma.

Our goal was to study the plasma concentration of ANV in patients who had had an early infarct, and to analyze its relationship to cardiovascular risk factors and the presence of APA and other hematological markers.

PATIENTS AND METHODS

Patients

We studied 62 consecutive patients from periodic follow-up in our practice (60 men and 2 women with a mean age of 47.7 years±5.9 years) who had had a myocardial infarct before the age of 45 years. The study exclusion criteria were: a) surgery, or acute infection or inflammatory disease within the last 3 months; b) neoplastic disease; c) being a recipient of anticoagulant therapy during the last year; d) angina, hemodynamic instability or deterioration in functional class during the 3 months prior to the study; e) MI or cardiac revascularization during the year prior to the study; f) permanent or paroxysmal atrial fibrillation; and g) greater than moderate valvulopathy. We recorded cardiovascular risk factors for all patients. We used 23 healthy volunteers as a control group who were of similar sex and age and who worked in the hospital; the individuals in this group did not have cardiovascular history but did have classic cardiovascular risk factors at the same rate as the study patients.

Methods

Blood samples were taken early in the morning, after at least 12-hours of fasting and a 20-minute rest. The blood was drawn a traumatically by trained staff who used syringes that were preloaded with trisodium citrate (0.011 mol/L, final concentration). The plasma, which was low in platelets, was obtained by centrifugation at 4°C and 2200 g for 20 minutes, and stored at –20ºC for later processing.

ABBREVIATIONS

MI: myocardial infarct.
ANV: annexin V.
APA: antiphospholipid antibodies.
ELISA: enzyme-linked immunosorbent assay.
t-PA: tissue plasminogen activator.
PAI-1: t-PA inhibitor.

APPA: antiphospholipid antibodies.
EPA: antiphospholipid antibodies.
ANV: annexin V.
ELISA: enzyme-linked immunosorbent assay.
t-PA: tissue plasminogen activator.
PAI-1: t-PA inhibitor.

References

We determined the plasma ANV concentration by enzyme-linked immunosorbent assay (ELISA) (Annexin V, Diagnostica STAGO, France). We studied the presence of APA, anti-β2 glycoprotein I, anti-β2 glycoprotein I/phospholipids complexes, and anti-ANV antibodies, using ELISA assay (anti-phospholipid, anti-β2 glycoprotein I, anti-β2 glycoprotein I/phospholipids complexes and anti-annexin V antibodies, Diagnostica STAGO, France). We determined the presence of lupus anticoagulant by the platelet neutralization procedure (Staclot PNP, Diagnostica STAGO, France).

We also studied the concentration of tissue plasminogen activator (t-PA) and its antigenic inhibitors (PAI-1) using the ELISA technique (Asserachrom kit, Boehringer-Mannheim, Germany). We determined the plasma concentration of the von Willebrand factor with the automated coagulometry immunological technique STA4 (LIA-VW test, Boehringer-Mannheim, Germany). We analyzed the plasma value of fibrinogen using the von Claus method (Boehringer-Mannheim, Germany). We determined the total cholesterol, HDL cholesterol, and triglyceride values with enzyme testing. The LDL cholesterol values were estimated with the Friedewald formula.

### Statistical analysis

We studied whether the variables analyzed followed a normal distribution with the Kolmogorov-Smirnov test. The ANV concentration did not follow a normal distribution pattern, and its value was expressed as median (25th and 75th percentiles). The discrete variables were expressed in percentages. To study the association between a continuous variable and a discrete variable we used the U Mann-Whitney test. The correlation between 2 quantitative variables was determined with the Spearman rank correlation test. To study the association between two discrete variables we used the χ² test. A value of $P<.05$ was considered significant.

### RESULTS

Patient and control characteristics are shown in Table 1.

The group of patients who had an early infarct had ANV values that were lower than the control group: 0.640 ng/mL (0.520 to 0.818) vs 1.570 ng/mL (1.140 to 2.390), $P<.01$ (Figure 1). We only found 2 patients with an IgG that was positive for anti-β2 glycoprotein I/phospholipids and other complexes with a positive IgG for anti-ANV antibodies. We did not find any patients who were positive for APA or anti-β2 glycoprotein I antibodies. In the control group, only 1 patient presented with APA and anti-β2 glycoprotein I antibodies. None of the patients or controls had a positive result from the lupus anticoagulant test. The 2 patients who tested positive for anti-ANV antibodies had ANV plasma values of 0.55 and 0.64 ng/mL, respectively, similar to the average found in the group overall.

Patient analysis data is summarized in Table 2. We observed a statistically significant, although a weak, correlation between the age of the patients and the ANV value ($r=.27; P<.05$). We did not find an association between ANV and the presence of various cardiovascular risk factors analyzed (smoking, arterial hypertension, diabetes mellitus, and dyslipemia). We did not find a statistically significant correlation between ANV values and lipid profiles. The plasma ANV values did not correlate significantly with the concentrations of antigenic t-PA or PAI-1. We also did not find a correlation with the von Willebrand factor or with fibrinogen values.

| TABLE 1. Summary of the demographic and clinical data of patients and controls |
|---------------------------------|----------------------------|-----------|
| Demographic data                | Patients (n=62)            | Controls (n=23) | $P$ |
| Age, years                      | 47.7±5.9                   | 44.5±6.1     | NS  |
| Sex, men/women                  | 60/2                       | 21/2        | NS  |
| Diabetes                        | 9                          | 4           | NS  |
| Hypertension                    | 17                         | 7           | NS  |
| Smoking                         | 13                         | 8           | 0.19|
| Dyslipemia                      | 35                         | 10          | NS  |

NS indicates not statistically significant, $P>.20.$
TABLE 2. Summary of the analytical data from patients with premature myocardial infarction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annexin V, ng/mL</td>
<td>0.640 (0.520-0.818)</td>
</tr>
<tr>
<td>Antigenic t-PA, ng/mL</td>
<td>14.6 (11.7-16.2)</td>
</tr>
<tr>
<td>Antigenic PAI-1, ng/mL</td>
<td>66.5 (33.9-94.2)</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>315.1–98.5</td>
</tr>
<tr>
<td>von Willibrand factor, %</td>
<td>109.6–32.6</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>227.9–75.3</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>45.2–11.8</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>149.3–65.4</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>142 (108-194)</td>
</tr>
</tbody>
</table>

Values are expressed as mean±standard deviation except for the ANV values, t-PA, PAI-1, and triglycerides which, because they did not follow a normal distribution pattern, are expressed as averages with the 25th and 75th percentiles shown in parentheses. t-PA indicates tissue plasminogen activator; PAI-1, tissue plasminogen activator inhibitor; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

DISCUSSION

There are few studies that have examined ANV values in MI, but elevated ANV concentrations have been described in the acute phase of infarct, and appear to normalize within a few hours. In our study, we found decreased concentrations of ANV in those patients who had a premature MI, after the acute event passed, which could lead to a state of hypercoagulability. ANV is a potent antithrombotic molecule because, thanks to its affinity for negatively charged phospholipids, it is capable of inhibiting prothrombin and X-asa complexes and reducing platelet adhesion and aggregation.

ANV possesses a high affinity for apoptotic cells, thanks to the fact that these produce a large number of phospholipids, particularly phosphatidylinerine, as has been shown in various studies both in vitro and in vivo. In addition to serving as a marker for the phagocyte cells to eliminate apoptotic cells, phosphatidylinerine plays a vital role in the initial phases of coagulation, as it increases the activity of the tissue factor/factor VIIa complexes. An increase in tissue factor expression in human atherosclerotic plaques has been shown to have an important role in the thrombogenicity of the plaque. The phosphatidylinerine produced by the apoptotic smooth muscle cells present in atherosclerotic plaque may regulate tissue factor activity; therefore, when ANV binds to it, thrombogenicity may be reduced. This fact suggests that ANV binding to apoptotic cells by means of phosphatidylinosine may be one of the causes of the low concentration of ANV found in our patients. In contradiction to this theory is the fact that normal ANV values have been described in groups of patients with MI antecedents who were not selected for their young age.

It has been proposed that APA may cause thrombotic events by means of the displacement of ANV in the procoagulant cell surfaces. New experimental data support the lack of association between ANV and antiphospholipid syndrome. Thus, it has been proven that the high affinity of ANV for binding to the surface of membranes does not appear to be affected by the presence of anti-β2 glycoprotein I antibodies. It has been documented that prothrombin activity is not affected when APA and ANV are present; of even greater interest is the fact that ANV has been shown to be capable of displacing APA. Therefore, the potential role of the low titers found in our patients does not appear to be related to the presence of APA. In addition, in our series, we found a very low prevalence of APA or related antibodies. The APA were not constantly present in the plasma time of patients, and it has even been suggested that detecting it may be more an epiphenomenon than an independent risk factor in patients with recurrent thrombotic events. Thus, in a cohort study, following a multivariate analysis adjusted for typical cardiovascular risk factors, the presence of APA was not an independent risk factor for death, a new infarct, or nonhemorrhagic ictus.

Our group has recently shown how polymorphism in the annexin V Kozak sequence (–1c>T) which occurs frequently in the Mediterranean population, produces higher translation efficacy, increasing protein synthesis capacity by nearly 6-fold. A study of the genotype in 166 patients who had an early infarct showed that the percentage of patients with mutant allele was significantly lower than in the general population studied. Multivariate analysis, after adjustment for sex, smoking, arterial hypertension, diabetes mellitus, and dyslipidemia, showed that this polymorphism has an independent protective effect for the development of a premature myocardial infarct. These findings have been confirmed in patients with cerebrovascular disease.

The lack of an association with classic cardiovascular risk factors, as well as the absence of a statistically significant association with other markers studied, strongly suggests a genetic basis for the concentration of plasma ANV.

We present the first study that analyzes the concentration of ANV in young patients who have had an infarct. Our data suggest that the low ANV plasma values in patients with premature AMI may indicate the existence of a hypercoagulable state that does not appear to be related to the antiphospholipid syndrome.

We cannot rule out the existence of a skew in patient selection in our study, as we have only studied patients who have survived an infarct. A more extensive series would be needed to confirm the suggested relationship between the genotype and phenotype and would deepen the physiopathological role of this potent antithrombotic molecule.
ACKNOWLEDGMENT

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REFERENCES