Clinical Research

β1-Selective Adrenoceptor Antagonists Increase Plasma Levels of Anti-p2β Antibodies and Decrease Cardiac Involvement in Chronic Progressive Chagas Heart Disease

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See editorial by McLean and Oudit, pages 267-269 of this issue.

ABSTRACT

Background: Studies indicate that antibodies cross-reacting with cardiac β1 adrenergic receptors are likely to play a role in the development of chronic Chagas heart disease (CCHD). In parallel, clinical trials have shown that β1 antagonists do not benefit patients with CCHD. In a group of patients with CCHD undergoing therapy with β-blockers, we have evaluated the levels of anti-p2β antibodies and the severity of CCHD.

Methods: We performed a cross-sectional study in Trypanosoma cruzi seropositive patients categorized according to a standard CCHD classification. We described anti-p2β antibodies were shown to play a pathogenic role in the development of heart tissue lesions such as electrocardiographic abnormalities and myocardial fibrosis, in humans and in animal models. The antigenic epitope present on the C-terminal end of the p2β, named R13 (IEEEDDGMGFLFD), bears similarity to the AEDSE acidic motif on the second extracellular loop of the β1-adrenergic receptor (AR). In vitro studies showed that the anti-p2β antibodies bind to the β1-AR, inducing an agonist response which is interrupted by cardio-selective β-blocker drugs such as bisoprolol. Furthermore, several clinical trials suggest that selective β1-blockers have beneficial effects on survival and on the quality of life of patients with CCHD. No studies have yet ascertained whether administration of β-blockers coexists with changes in the levels of anti-p2β antibodies in patients with CCHD. To ascertain that issue the present study was undertaken.

Methods

Study population and subject evaluation

We performed a cross-sectional study in 80 adult patients yielding positive serology results for T. cruzi according to the recommendation of the World Health Organisation. They were recruited at the Internal Medicine Service of the J.B.
sification. All individuals were subjected to a complete clinical examination.

Results: There was no association between CCHD stages, electrocardiographic conduction disturbances, and echocardiographic pathological signs with the levels of autoantibodies. However, when patients were analyzed according to selective cardio-β-blocker therapy, those receiving treatment had higher levels of anti-p23. Patients from CCHD stage III treated with combined therapy of cardio-β-selective blockers, enalapril, and statins, presented decreased cardiac involvement and lower score of risk of mortality than individuals from the same group who were not treated.

Conclusions: Our results suggest that selective cardio-β-blockers might modify the autoantibody anti-p23 levels, and that combined therapy in patients with stage III CCHD might be associated with lower cardiac involvement and risk score of mortality in patients with heart failure. Longitudinal studies will help to ascertain the proper role of β-blockers in the immunopathological processes underlying chronic Chagas disease.

Méthodes: Nous avons réalisé une étude transversale chez des patients séropositifs pour le Trypanosoma cruzi répartis selon une classification standard de la CCC. Tous les individus étaient sujets à un examen clinique complet.

Résultats: Il n’y avait aucune association entre les stades de la CCC, les perturbations de la conduction électrocardiographique et les signes pathologiques à l’echocardiogramme quant aux concentratons d’autoanticorps. Cependant, lorsque les patients étaient analysés selon le traitement par β-bloquants cardioselectifs, ceux recevant le traitement avaient des concentrations plus élevées d’anti-p23. Les patients ayant une CCC de stade III, qui reçoivent le traitement combiné de β-bloquants cardioselectifs, d’enalapril et de statines, montraient une diminution de l’implication cardiaque et un plus faible score de risque de mortalité que les individus du même groupe qui n’étaient pas traités.

Conclusions: Nos résultats suggèrent que les β-bloquants cardioselectifs pourraient modifier les concentrations d’anticorps anti-H2 et que le traitement combiné chez les patients ayant une CCC de stade III pourrait être associé à une implication cardiaque et un score de risque de mortalité plus faible que chez les patients ayant une insuffisance cardiaque. Des études longitudinales aideront à établir le rôle propre des β-bloquants dans les processus immunopathologiques de la maladie de Chagas chronique sous-jacentes.

Iturraspe Hospital, Santa Fe, Argentina, and categorized into 3 groups according to the CCHD classification provided by Sorzio et al. 36 Sampled individuals were subjected to a complete clinical examination including electrocardiogram, chest and abdominal x-ray, echo Doppler cardiography, and biochemical tests. Patients with 2 or more risk factors for coronary artery disease or history of other cardiac diseases, renal disturbances, thyroid disease, or any other systemic complaints, and treatment with anti-T. cruzi compounds or immunosuppressive drugs were excluded. The study received ethical approval from the Ethics Review Board of Universidad Nacional del Litoral. Informed consent was obtained from all patients.

According to the CCHD classification, the sampled individuals were grouped into: (1) 30 individuals with CCHD stage I, indeterminate phase; (2) 20 patients with CCHD stage II (ie, electrocardiographic conduction disturbances); and (3) 30 patients with CCHD stage III (ie, clinical heart failure and/ or showing dilated cardiomyopathy using echo Doppler cardiography); within this group there were 16 individuals with a recent diagnosis of CCHD without any treatment. Clinical heart failure was defined according to the guidelines for the diagnosis and treatment of acute and chronic heart failure from the European Society of Cardiology. 31

The Risk Score for Predicting Death in Chagas Heart Disease 22,23 was applied to all patients.

Based on the study purposes, patients were also classified depending on whether they were receiving therapy with cardio-β-selective blockers, or not. Patients receiving treatment with cardio-β-selective blockers had been taking the drugs for the 6 previous months, as a minimum, at doses ranging from 5 to 10 mg/d for bisoprolol or 50 to 100 mg/d for azenol.

Homogenate production and protein purification

Epimastigotes of T. cruzi (Tahuatén strain) were grown in liver infusion tryptose medium supplemented with 10% fetal calf serum (Cultilab, São Paulo, Brazil) to produce homogenerate. Total homogenates of epimastigotes were obtained by resuspension of the washed cells in 5 volumes of 1 mM Na2-2-proto-1-lysine chloroethyl ketone and 1 mM phenylmethylsulfonyl fluoride in distilled water, frozen and thawed (4 cycles), and sonication (20 kHz, 30 W, 2 minutes). 30

Escherichia coli BL21 (DE3) cells bearing the plasmidic constructions pET-32α/p23 and flagellar repetitive antigen (FRA) were grown overnight in Luria-Bertani medium, supplemented with 0.1 mg mL/L ampicillin at 37 °C, with agitation. Protein expression was induced in 1 mM isopropyl-β-D-thiogalactopyranoside and purified using an Ni-Nitrilotriacetic acid column (GE), as described elsewhere. 31

Measurement of antibodies

Antibodies were measured using immunoassay (ELISA) according to standard procedures. 29 Briefly, microtiter plates were coated with 0.5 μg of specific antigens (p23, FRA, and homogenerate parasites) in 0.05 M carbonate-bicarbonate buffer, pH 9.6, and incubated overnight at 4 °C. After the plates were washed thrice with 0.01% Tween in phosphate-buffered saline, they were blocked with 5% bovine serum albumin and incubated with a 1:100 dilution of human serum. Microplates thus sensitized were incubated with a 1:100 dilution of human serum in 1% skimmed milk in phosphate-buffered saline at 37 °C for 60 minutes. The plates were washed and peroxidase-conjugated goat anti-human immunoglobulin G (Sigma, St Louis, MO) was added. Plates were read at 450 nm in an ELISA reader (Maxiplate Microplate Reader; Invitrogen, Carlsbad, CA) after incubation with trimethylbenzidine in H2O2. For each specific antigen, the results were expressed as the mean of the optical density (OD) of 2 simultaneous assessments of the same serum sample. In each plate and for each specific antigen, 6 negative control samples (from healthy individuals seronegative for
were assayed simultaneously. ELISA negative standard cutoff values were calculated as the mean OD of the negative serum samples plus 2 SDs. The levels of antibodies were expressed as the ratio between the OD of the sample and the OD of the negative standard cutoff. This index is referred to as the IODN (index of the OD of autoantibodies in relation to the negative control). An IODN ≤ 1 was considered negative. Anti-p2β sensitivity and specificity was 99%. Interassay and intra-assay variability was 1.7 and 1.2, respectively.

Statistical analysis

Data were analyzed using MedCalc version 12.2.1. Normal distributions of the continuous variables were tested using the Kolmogorov-Smirnov method. The data are expressed as mean ± SD or median and interquartile range. Groups were compared in relation to age, antibody levels, and CCHD stages. χ² test or Fisher exact were used for categorical variables, and the 1-way analysis of variance (ANOVA) (Student Newman-Keuls post hoc test for all pairwise comparisons) was used to compare means of IODN values and age among the groups defined according to CCHD stage. A P value < 0.05 was considered significant.

Results

General population

The mean age of sampled individuals was 52.85 ± 2.18 years. There were no between-group differences as for sex distribution, but CCHD stage III patients were older than the remaining ones (P < 0.05). Age was not associated with any chronic Chagas disease stage and presented no correlation with tested antibodies.

There were 40 patients with essential hypertension receiving treatment at the time of inclusion, 11, 15, and 14 cases from CCHD stage I, CCHD stage II, and CCHD stage III, respectively. The number of individuals with hypertension from the CCHD stage II group was greater than from the CCHD I group (P = 0.01), but not from the CCHD III group (P = 0.09). Individuals from CCHD stages I and II groups were receiving monotherapy treatment (8 with angiotensin-converting enzyme inhibitors and 18 with cardioselective β1-blockers). All cases of CCHD stage III with hypertension were being treated with combined therapy of enalapril plus β-adrenergic antagonist drugs. Systolic blood pressure in stage I individuals was significantly lower than values seen in the remaining groups (ANOVA F-ratio, 14.84; P < 0.001; Student Newman-Keuls test for all pairwise comparisons, P < 0.05).

The median time of therapy for the 3 patient groups was 6.3, 6.9, and 7.2 years, respectively (not significant). There was no correlation between the length of therapy and the level of autoantibodies.

The characteristics of the patients in each group and the distribution of the antibodies are summarized in Table 1.

Among patients from the CCHD stage III group, those with treatment presented decreased cardiac involvement according to echocardiography and lower risk score of mortality (Supplemental Table S1).

Electrocardiographic findings

The most frequent electrocardiographic alterations in the CCHD stage II and III groups were left anterior fascicular block with right bundle branch block (n = 30), atrial fibrillation (n = 8), and ventricular extrastoles (n = 4). There was no association of electrocardiographic conduction disturbances and hypertension with IODN values for anti-p2β, anti-FRA, and antiparastite homogenates. Patients receiving β-blockers presented lower heart rate and higher levels of anti-p2β than those without treatment. Only 2 patients taking bisoprolol had a prolonged QRS complex.

Table 1. Characteristics of CCHD patients according to group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I (n = 30)</td>
<td>II (n = 26)</td>
</tr>
<tr>
<td>Age, y</td>
<td>41.8 ± 12</td>
<td>54.2 ± 11.3</td>
</tr>
<tr>
<td>Sex, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>119 ± 10</td>
<td>130 ± 10</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>78 ± 10</td>
<td>85 ± 8</td>
</tr>
<tr>
<td>Risk score of death, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>Low risk</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>Heart failure, n</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IODN-p2β</td>
<td>5.57 ± 1.995</td>
<td>5.727 ± 2.636</td>
</tr>
<tr>
<td>IODN-FRA</td>
<td>5.547 ± 2.525</td>
<td>5.052 ± 2.366</td>
</tr>
<tr>
<td>IODN-parasite homogenate</td>
<td>7.807 ± 2.434</td>
<td>6.937 ± 2.897</td>
</tr>
</tbody>
</table>

Quantitative variables are expressed as mean ± SD.
CCHD, chronic Chagas heart disease; FRA, flagellar repetitive antigen; IODN, index of the optical density of autoantibodies in relation to the negative control; NS, not significant.
† CCHD group III different from CCHD groups I and II.
‡ CCHD group I different from CCHD groups II and III.
§ CCHD group III different from CCHD groups I and II; CCHD groups II and III different from CCHD group I.
∥ CCHD group III different from CCHD groups I and II.
Echocardiogram findings

The echocardiograms were performed according to the recommendations of Acquafra et al.\(^5\) Twelve patients with moderate to severe cardiac impairment presented left ventricular apical aneurysms. Two individuals from the CCHD II group had apical septal hypokinesia without diastolic or systolic disturbance, and 2 patients had concentric ventricular hypertrophy. Among the individuals with CCHD stage III: (1) 20 of them had globally dilated cardiomyopathy with reduced ejection fraction; (2) 6 had heart failure with preserved ejection fraction, left atrial enlargement with concentric ventricular hypertrophy and abnormal left ventricular relaxation; and (3) 4 had heart failure with preserved ejection fraction and abnormal left ventricular relaxation.

Antibodies

All patients presented detectable levels of anti-p2β, anti-FRA, and antiparasite homogenates. Comparison of autoantibodies revealed no differences either according to CCHD group or risk score of mortality. Autoantibody values were unrelated to age, sex, and comorbidities such as hypertension or overweight.

To assess whether treatments modified autoantibody levels, patients were next grouped as follows: (1) without treatment (n = 46); (2) enalapril monotherapy (n = 8); (3) β1-blocker monotherapy (n = 18) and combined treatment with cardio-β1-selective blockers, enalapril, and atenolol (n = 14). Enalapril was not correlated with any variation of autoantibodies levels. Conversely, patients treated with selective cardio-β1-blockers (atenolol or bisoprolol) and with combined treatment presented the highest levels of anti-p2β (ANOVA F-ratio, 7.34; P < 0.001; Student Newman-Keuls test for all pairwise comparisons, P < 0.05), but not for anti-FRA and antiparasite homogenates. There was no statistical difference in anti-p2β values between the combined treatment and β1-blocker monotherapy recipients (Fig. 1).

Further comparisons on the levels of anti-p2β for each CCHD stage according to cardio-selective β1 antagonist drug intake (groups CCHD stage I and II) or combined therapy (CCHD stage III group), revealed that treated individuals had higher values of anti-p2β either in general (P = 0.016) or for every subgroup analysis (P < 0.05 Student Newman-Keuls test) (Supplemental Table S1).

Discussion

It has been suggested that the autoimmune response against myocardial antigens might play a role in the development of the severe forms of CCHD.\(^1\) Among the several autoantibodies that have been described, anti-p2β, anti-B13, and antimiscarriage (anti-M2) are likely to play a pathogenic role in the development of heart tissue lesions.\(^2\) Matsui et al.\(^1\) found, in an experimental model, that immunization with synthetic peptides corresponding to the anti-p2β or anti-M2 induced cardiac morphological changes resembling dilated cardiomyopathy. In contrast, levels of anti-p2β and anti-M2 antibodies did not correlate with the severity of CCHD,\(^4\) although some evidence for a marker of cardiac impairment was recently provided in an analysis of anti-B13 antibodies\(^4\) and matrix metalloproteinases 2 and 9.\(^5\) Beyond these facts, presence of autoimmune antibodies might be fueled by parasite persistence, because specific treatment for Chagas disease with benznidazole reduced the anti-p2β titers.\(^6\,9\)

Turning to anti-p2β, Elies et al.\(^1\) observed that these autoantibodies bind to the β1-AR on myocytes and induce an agonist response that is completely blocked by the β1-selective antagonist bisoprolol. Considering that anti-p2β autoantibodies are implicated in myocyte apoptosis and/or electrocardiogram conduction abnormalities by binding to the cardiac β1-AR, β1-selective antagonist therapy might be useful to prevent the development of CCHD. Issa et al.\(^1\) performed a clinical trial, studying the influence of β-blockers in patients with CCHD, and observed that these drugs improved the prognosis and survival of such patients. Also, in another study, β-blocker therapy was associated with a favorable outcome in patients with chronic heart failure secondary to CCHD.\(^1\) However, none of these clinical trials evaluated whether the beneficial effects of β-blockers were related to a variation of the autoantibody levels. As already mentioned, anti-p2β antibodies were assessed in chagasic patients from different disease stages in relation to cardiac involvement.\(^1\) Both studies found no correlation between CCHD groups and antibody levels. However, the studies did not explore whether cardio-selective β1-blockers were associated with anti-p2β variation. As demonstrated here, all patients presented circulating anti-p2β antibodies unrelated to clinical manifestations of CCHD, but their levels were increased in individuals treated with β1-blocker drugs or combined therapy with cardio-β1-selective blockers, enalapril, and atenolol, or CCHD stage III cases. In light of our results, the reported weak correlation between cardiac involvement and serum levels of p2β might be explained because these antibodies are complexed with the β1-ARs in heart tissue.
Considering that β1-antagonist drugs compete with the autoantibody p2β for the binding site of the β1-adrenergic cardiac receptors, this might be responsible for the increased levels of autoantibody p2β in patients receiving treatment with β1-blocker drugs, reported here. Other important result of our work is that treated patients of the CCHD stage III group presented higher values of anti-p2β associated with decreased cardiac involvement according to echocardiography and lower risk score of mortality with respect to individuals not treated from the same group. Although cardio-selective β1-antagonist drugs have been correlated with better outcome of patients with CCHD with heart failure, enalapril was also associated with improvement of heart failure in CCHD.

Limitation of the study

This was a single-centre study with a relatively reduced sample size. The nature of this cross-sectional study renders it difficult to infer how β1-blockers might change antibody anti-β1 levels over time, and the clinical progression.

Conclusions

Our results are suggestive of a role of selective cardio-β1-blockers in modifying the autoantibody anti-p2β levels, and that combined therapy in patients with CCHD stage III might be associated with lower cardiac involvement and risk score of mortality in patients with heart failure. Longitudinal studies will help to ascertain the proper role of β1-blockers in the immunopathological processes underlying chronic Chagas disease.

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Disclosures

The authors have no conflicts of interest to disclose.

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Supplementary Material

To access the supplementary material accompanying this article, visit the online version of the Canadian Journal of Cardiology at www.elsevier.com and at http://dx.doi.org/10.1016/j.cjca.2013.08.017.