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Anticipating the side effects of benznidazole: HLA-B*35 and patch test

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ABSTRACT

Introduction: Treatment of Chagas disease frequently causes distress to patients due to a high incidence of adverse effects. Different preemptive tests have been researched to prevent these effects and to allow focus to be given to certain predisposed patients. Benznidazole is the most prescribed Chagas disease treatment in Spain.

In this work, we analyzed the genetic markers HLA-B*35 allele group and HLA-B*35:05 allele specifically, as well as an allergy patch test, as benznidazole's most frequent adverse effects are cutaneous.

Methods: HLA-B intermediate-resolution genotyping was performed followed by a high-resolution level analysis. Cutaneous allergies were tested using strips impregnated with a mixture of benznidazole and placed on the upper back of patients before starting treatment.

Results: In our sample of more than 400 patients, there was almost no relationship between any kind of side effect and either of the HLA-B alleles studied. The patch testing was quickly discarded as a preemptive test due to its low sensitivity (16.7%).

Conclusion: In conclusion, we were unable to replicate and corroborate genetic markers identified by other groups and there is currently no test that can anticipate the adverse effects of benznidazole, therefore, more investigation should be carried out in this field.

Anticipándonos a los efectos adversos del benznidazol: HLA-B*35 y *patch test*

Palabras clave:
Benznidazol
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HLA-B*35:05
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RESUMEN

Introducción: El tratamiento de la enfermedad de Chagas causa frecuentemente angustia a los pacientes debido a su alta incidencia de reacciones adversas. Se han investigado algunos test para anticiparnos y poner especial foco en aquellos pacientes susceptibles de sufrir estos efectos. Benznidazol es el tratamiento más prescrito para la enfermedad de Chagas en España.

En este trabajo hemos analizado la familia alélica HLA-B*35, y específicamente HLA-B*35:05, así como un test alérgico epicutáneo *(patch test)*, teniendo en cuenta que el efecto adverso más frecuente son reacciones cutáneas.

Métodos: El HLA-B de los pacientes se genotipó con resolución intermedia y después se analizó mediante alta resolución. Antes del tratamiento, se testó una posible hipersensibilidad cutánea mediante tiras impregnadas con una solución de benznidazol adheridas en la parte alta de la espalda de los pacientes.

Resultados: En nuestra muestra de más de 400 pacientes no se detectó relación entre ningún tipo de efecto adverso y ningún alelo de HLA-B estudiado. La prueba de *patch test* se canceló al poco de empezar el estudio debido a su baja sensibilidad (16,7%).

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Conclusión: En conclusión, no fuimos capaces de replicar el resultado obtenido por otros grupos que nos permitiera corroborar este marcador HLA-B como predictor de efectos adversos, por lo que actualmente no existe ningún test que nos permita anticiparnos a los efectos adversos del benznidazol, y debería investigarse más en esta línea.

Introduction

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, affects 6–7 million people worldwide.[1] Treatment with benznidazole or nifurtimox is strongly recommended in the acute phase as well as in congenital disease, reactivations, and chronic disease in children under 18 years old.[2] Although the efficacy of treatment in chronic adults is questioned, a prescription is generally offered due to the absence of alternative treatment options.[3–5] The major complications for universal treatment are the side effects, being suffered by ∼45–92% of patients in several studies.[6–8] Even though benznidazole is considered to be better tolerated, both drugs cause a broad spectrum of complications.[6,9–11] Being able to anticipate these effects, either by giving specific drugs, by testing for cutaneous allergy, or by finding genetically more predisposed individuals, could improve tolerance. Studies based on preemptive methods are scarce; however, Salvador et al. proposed the HLA-B*35:05 allele as a possible genetic marker related to skin reactions in benznidazole treated patients.[12] A few years later, Bosch-Nicolau et al. studied a larger patient group, rejecting the specific allele HLA-B*35:05 and instead associating HLA-B*35 group.[13] Structurally, HLA-B*35:05 belongs to the group of HLA-B*35 alleles.

In the last two decades, some breakthrough has been made relating genetics and drug adverse reactions.[14–16] The HLA gene complex encodes a group of proteins that are expressed in all types of body cells and are responsible for the adaptative immunogenicity to antigens. They are also the most polymorphic genes of the human genome and can be involved in how the immune response is delivered by certain individuals or groups.[17,18] Three mechanisms have been proposed in order to explain how small molecule drugs elicit T-cell-mediated hypersensitivity responses: p.i. concept (T-cells recognize immunogenic drug-HLA complexes), hapten–prohapten concept (body proteins/peptides are modified by the drug or its metabolites and the new epitopes are processed as foreign) and the altered repertoire (HLA antigen-binding cleft is modified by the drug altering the recognition pattern).[19] Some successful links have been stablished among certain drug adverse reactions and specific HLA alleles, such as abacavir/HLA-B*57:01 (altered repertoire), allopurinol/HLA-B*58:01 (p.i. concept), carbamazepine/HLA-B*15:02 (p.i. concept) or flucloxacillin/HLA-B*57:01 (hapten/pro-hapten concept).[19,20]

Our aim in this work is to evaluate HLA-B*35 allele group and the specific HLA-B*35:05 allele as side effect prognostic markers in recently diagnosed Chagas disease patients in our Tropical Medicine Outpatient Clinic. The second objective is to evaluate a preemptive benznidazole allergy test as a predictive marker.

Materials and methods

This cross-sectional study with sequential recruitment was performed in our Tropical Outpatients Clinic, located in a third-level hospital in the southeast of a non-endemic country, we diagnose Chagas disease using two complementary methods: a chemiluminescent microparticle immunoassay (ARCHITECT Chagas®, Abbott) and an indirect immunofluorescence assay (Inmunofluor Chagas kit, Biocientifica S.A.), and offer treatment to all patients based on the WHO guidelines.[1] From August 2015 to December 2018, 611 adult patients were offered benznidazole as a starting treatment. The prescribed dosage was 5–7.5 mg/kg divided into three daily doses for 60 days.[2] Patients were enrolled in the study protocol at the medical visit, when Chagas Disease diagnoses was reported and specific treatment was offered. Informed Consent was also explained and signed. Peripheral blood samples were taken for HLA-B testing, as long as an Allergy Outpatients Clinic appointment for a patch test analysis was scheduled.

The definition of adverse effects was based on the Common Terminology Criteria for Adverse Events, version 4.0.[21] Adverse effects that could not be included in any category were considered as "Others".

The inclusion criteria comprehended every patient diagnosed in our Clinic who had completed a benznidazole treatment, had their HLA-B alleles typed, patch testing done and had returned for follow-up after treatment. The exclusion criteria included pregnant women, patients under 18 years of age, those who refused to undergo the HLA-B alleles and/or patch testing, patients who did not return for follow-up, and those without treatment adherence.

This study has been approved by the Clinical Research Ethics Committee of Virgen de la Arrixaca Clinical University Hospital and by the Research Ethics Committee of Murcia University in 2018 and updated in 2020.

HLA-B alleles typing

DNA was extracted from peripheral blood samples using the QIAsymphony® DNA Mini kit (Qiagen, Dusseldorf, Germany). HLA-B intermediate-resolution genotyping was undertaken using the LABType CWD Class I B Typing Test (One Lambda, West Hills, CA) and analyzed by Luminex® based LABScan 3D (One Lambda, West Hills, CA). On the other hand, HLA-B high-resolution typing was completed using the SBTexcellerator HLA-B core kit (GENDX) (Utrecht, Netherlands) and analyzed by SBTengine® (GENDX, Utrecht, Netherlands). Sequencing was performed with a 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). All alleles of B*35:05 found in our study were B*35:05:01:01 and were retyped by SBT technique to avoid ambiguities. We decided to notify only the high-resolution alleles obtained with Luminex technique.

Patch testing

To perform the patch test, the Hospital Pharmacy Service prepared a mixture of benznidazole (obtained by crushing pills) plus 20% petrolatum as contactant. In the Allergy Outpatients Clinic, one drop of the mixture was placed in an 8 mm Finn Chamber® on Scanpor® (SmartPractice) and the strip was applied to the patient's upper back with adhesive hypoallergenic tape. After a 48-h exposure, an initial reading was taken, followed by a second reading at 72 h and a third reading at 92 h. The results were interpreted according to the European Society of Contact Dermatitis guidelines.[22]

This procedure was withdrawn after most of the patients tested negative and the majority developed side effects.

Table 1
Country of origin.

Country	*N* (%)
Argentina	2 (0.5)
Bolivia	405 (97.4)
Brazil	2 (0.5)
Ecuador	6 (1.4)
Paraguay	1 (0.2)

N: number of patients.

Statistics

Continuous variables are presented as means, standard deviation, and minimums/maximums, and categorical data are presented as absolute frequencies and percentages.

Univariate logistic regression was carried out to assess the association between HLA-B*35:05 or HLA B*35 and withdrawal, suffering adverse effects, and several types of adverse effects, including skin reactions.

Statistical analysis was performed with the SPSS 25.0 program for Windows. A p-value < 0.05 was statistically significant.

Results

The final study sample, after exclusion criteria, included 416 patients, of whom 70.7% (294) were women. Patient age was ranged between 18 and 66 years old (mean = 39.6 [typical deviation = 9.2]). Bolivian was the most frequent nationality (Table 1).

The results showed that there is no association between HLA-B*35:05 allele or HLA-B*35 allele group and withdrawal, suffering adverse effects, or any of the adverse effects, including skin reactions, except for HLA-B*35 allele group being related to a protective effect against gastrointestinal disorders. Patients who did not develop gastrointestinal disorders had 2.5 times more possibilities to have HLA-B*35 allele group (Tables 2 and 3).

Regarding patch testing, the procedure was carried out in 38 cases, of which only 4 patients were positive. Among the negative patients, 20 developed adverse effects during treatment (58.8%), 18 of whom developed skin disorders classified by severity as 6 mild, 10 moderate, and 2 severe. Of the 4 patients with positive results in the patch test, all developed adverse effects (moderate and severe skin reactions). Therefore, the Positive Predictive Value (PPV) was 100% and the Negative Predictive Value (NPV) was 41.18%.

Discussion

The treatment of Chagas disease is a global concern, and several research teams are nowadays working on identifying drugs that are better tolerated than the approved ones.[23–25] The best acceptable choice in our region, benznidazole, still has many adverse effects. Among them, skin reactions are the only reactions shown to be related to the discontinuation of treatment.[6] Because of this, it is of major interest among clinicians to be able to predict individuals who are prone to develop these reactions.

Some authors have reported that genes may have a role in the association among BZN and its adverse reactions.[16,26] Salvador et al. and Bosch-Nicolau et al. published some breakthrough research in this line of work, linking specific genetic markers (HLA-B*35:05 allele and HLA-B*35 allele group) to benznidazole skin reactions.[12,13] Among Bolivians however, HLA-B*35 is the most frequent HLA-B allele group.[26–30] Considering the latter, a risk of bias cannot be omitted. In our study, which included a larger cohort of cases, we were not able to replicate their results, even taking into account that the great majority of the patients were also Bolivians. This could be due to an increment of HLA-B possibilities with increasing sample size. Only the association of having any allele of HLA-B*35 group with an absence of gastrointestinal disorders appears as a possible protective effect. There are no other data in the literature about this possible relationship and we cannot give any further explanation because it might be the result of chance.

In the case of patch testing, our results prevent it from being established as a preemptive strategy because of its low sensitivity (16.7%), despite its high specificity (100%).

Limitations in our study are the high prevalence of women (which have been previously reported as more prone to suffer from adverse reactions with BZN treatment[26,32,33]) and the lack of the

Table 2
Association between adverse effects and treatment fulfillment with the presence of HLA-B*35:05 allele.

	HLA-B*35:05 (*N* [%])		Univariate	
	No	Yes	OR (CI 95%)	*p*-Value
Treatment fulfillment				
No	48 (75)	16 (25)	1	
Yes	253 (71.9)	99 (28.1)	1.17 (0.64–2.16)	0.607
Adverse effects				
No	134 (70.5)	56 (29.5)	1	
Yes	167 (73.9)	59 (26.1)	0.85 (0.55–1.30)	0.444
Skin disorders				
No	162 (71.7)	64 (28.3)	1	
Mild	74 (73.3)	27 (26.7)	0.92 (0.55–1.57)	0.768
Moderate/severe	65 (73)	24 (27)	0.94 (0.54–1.62)	0.81
Gastrointestinal disorders				
No	281 (71.9)	110 (28.1)	1	
Yes	20 (80)	5 (20)	0.64 (0.23–1.74)	0.382
Neurologic disorders				
No	269 (72.1)	104 (27.9)	1	
Yes	32 (74.4)	11 (25.6)	0.89 (0.43–1.83)	0.75
Other disorders				
No	277 (73.3)	101 (26.7)	1	
Yes	24 (63.2)	14 (36.8)	1.60 (0.80–3.21)	0.187

OR: odds ratio.
CI: confidence interval.
N: number of patients.

Table 3
Association between adverse effects and treatment fulfillment with the presence of HLA-B*35 allele group.

	HLA-B*35 (*N* [%])		Univariate	
	No	Yes	OR (CI 95%)	*p*-Value
Treatment fulfillment				
No	29 (45.3)	35 (54.7)	1	
Yes	167 (47.4)	185 (52.6)	0.92 (0.54–1.57)	0.753
Adverse effects				
No	85 (44.7)	105 (55.3)	1	
Yes	111 (49.1)	115 (50.9)	0.84 (0.57–1.24)	0.373
Skin disorders				
No	101 (44.7)	125 (55.3)	1	
Mild	50 (49.5)	51 (50.5)	0.82 (0.52–1.32)	0.42
Moderate/severe	45 (50.6)	44 (49.4)	0.79 (0.48–1.29)	0.347
Gastrointestinal disorders				
No	179 (45.8)	212 (54.2)	1	
Yes	17 (68)	8 (32)	0.40 (0.17–0.94)	**0.036**
Neurologic disorders				
No	181 (48.5)	192 (51.5)	1	
Yes	15 (34.9)	28 (65.1)	1.76 (0.91–3.40)	0.093
Other disorders				
No	178 (47.1)	200 (52.9)	1	
Yes	18 (47.4)	20 (52.6)	0.99 (0.51–1.93)	0.974

OR: odds ratio.
CI: confidence interval.
N: number of patients.
In bold, statistically significant values.

pre-study sample size calculation, factors that might decrease the reliability of the results.

In conclusion, it would be a great advance to have some kind of indicator to anticipate adverse effects in the treatment of Chagas disease, which are the biggest breaking point. Nevertheless, our attempts to replicate and corroborate genetic markers identified by other groups have been unsuccessful. Even so, being able to associate another type of HLA with adverse reactions is still a work in progress. Chagas disease is currently the focus of many research groups, and we expect outstanding developments soon.

Conflict of interests

The authors state that they have no conflict of interests.

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