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ORIGINAL ARTICLE

Correlation, in previously treated HIV-1 positive patients, between hypersensitivity reaction to abacavir and the presence of the HLA-B*5701 allele

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Abstract
Introduction: Hypersensitivity reaction to abacavir (a powerful inverse transcriptase inhibitor) is a serious adverse effect that limits its use in antiretroviral treatment and requires a high level of clinical surveillance. Certain haplotypes of the primary histocompatibility complex proteins (HLA-B*5701) are very significant predictors of the risk of hypersensitivity to this drug. The purpose of this study is to identify the cases where a probable hypersensitivity reaction to abacavir presented the HLA-B*5701 allele.
Method: A retrospective study was conducted in all HIV-1 positive adult patients infected treated with abacavir between January 2000 and December 2007, in Department 6 of the Agencia Valenciana de Salud (Valencia Health Agency). The adverse effects developed by the patients were collected to determine which cases presented a probable clinically diagnosed hypersensitivity reaction. Finally, these 39 patients were screened for HLA-B*5701.
Results: In total, 323 patients were treated with abacavir between 2000 and 2007. The treatment was discontinued in 12.1% (n=39 patients) presenting a hypersensitivity reaction. Nine (23.1%) of these were HLA-B*5701 positive. Eight patients presented skin rash and positivity was observed in only single patient with gastrointestinal symptoms and fever.
Conclusions: The administration of the HLA-B*5701 gene test may be of benefit in clinical practice, because it prevents diagnostic errors of the hypersensitivity reaction and enables more accurate interpretation of the symptoms.

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PALABRAS CLAVE
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Correlación, en pacientes infectados por el VIH-1 y previamente tratados, entre la reacción de hipersensibilidad a abacavir y el alelo HLA-B*5701

Resumen
Introducción: La reacción de hipersensibilidad a abacavir (un potente inhibidor de la transcriptasa inversa) es un efecto adverso importante que limita su uso en la terapia antirretroviral y precisa un elevado grado de vigilancia clínica. Determinados haplotipos de las proteínas del complejo principal de histocompatibilidad (HLA-B*5701) predicen, de forma muy significativa, el riesgo de hipersensibilidad a este fármaco. El objetivo del estudio es identificar los casos que, después de desarrollar una probable reacción de hipersensibilidad a abacavir, presentaban el alelo HLA-B*5701.
Métodos: Se ha realizado un estudio retrospectivo a todos los pacientes adultos infectados por el virus de la inmunodeficiencia humana 1 (VIH-1) que recibieron tratamiento con abacavir entre enero de 2000 y diciembre de 2007, en el Departamento 6 de la Agencia Valenciana de Salud. Se recogieron los efectos adversos desarrollados por los pacientes para identificar los casos con probable reacción de hipersensibilidad diagnosticada clínicamente. Finalmente, se realizó la tipificación de HLA-B*5701 a estos 39 pacientes.
Resultados: En total, 323 pacientes recibieron tratamiento con abacavir entre 2000 y 2007. Se retiró el tratamiento por reacción de hipersensibilidad a 39 pacientes (12,1%); 9 (23,1%) de ellos resultaron HLA-B*5701 positivo; 8 pacientes manifestaron exantema y únicamente se observó positividad en un paciente con síntomas gastrointestinales y fiebre.
Conclusiones: La realización del test genético HLA-B*5701 podría ser favorable para la práctica clínica habitual, ya que evita errores en el diagnóstico de la reacción de hipersensibilidad y permite interpretar los síntomas con más seguridad.

Introduction

Hypersensitivity reaction to abacavir is an important adverse effect that limits its use in antiretroviral treatment and requires a close monitoring.

Abacavir is a reverse transcriptase inhibitor recommended by GESIDA/Spanish National AIDS plan (*Plan Nacional sobre el Sida*) as one of the drugs of choice for beginning treatment of patients infected with human immunodeficiency virus (HIV).[1,2] It is available in a one-per-day dosage in combination with other antiretroviral agents. Regarding its toxicity profile, the Spanish Agency of Drugs and Healthcare Products (*Agencia Española de Medicamentos y Productos Sanitarios*, AEMPS) recently alerted us to the possible relationship between abacavir and didanosine with increased risk of myocardial infarction.[3,4]

The hypersensitivity reaction (HSR) appears mainly in Caucasian patients or those with a CD8 lymphocyte count >850 cells/mL at the start of treatment.[5] In general, symptoms appear in 5%-8% of patients in the first 6 weeks of treatment with abacavir (mean time to present is 11 days), although these reactions can occur at any moment during therapy.[6-8] It is characterised by the onset of fever and/or eruption as a part of the syndrome. Other frequently observed signs include gastrointestinal symptoms (nausea, vomiting, diarrhoea, and abdominal pain) and lethargy or general malaise. Hypersensitivity reactions have occurred without eruptions or fever. Other symptoms may include dyspnoea, rheumatic symptoms (myalgia, musculoskeletal pain), headache, paraesthesia, or oedema.

It is likely that intermittent treatment increases the risk of developing sensitivity, therefore increasing the risk of a clinically significant hypersensitivity reaction. Symptoms related with hypersensitivity reactions become worse if the treatment continues, and normally resolve if the treatment is discontinued. Continuing treatment with abacavir following a suspected hypersensitivity reaction is contraindicated, due to the increased risk of toxicity.

Various factors complicate diagnosis, including varying and unspecific signs and symptoms and the combination of abacavir with other antiretroviral drugs which may superimpose different adverse effect profiles.

The human species has a group of genes that are closely linked and very polymorphic with a large number of genetic variations (alleles) in each locus: this is the major histocompatibility complex (MHC), located in chromosome 6 and also known as the HLA region. The molecules that are codified by the MHC intervene in a centralised way in the development of specific immune responses.[9]

Due to the relationship between MHC proteins and the development of hypersensitivity reactions to drugs, there is a special interest in determining whether the haplotypes of the MHC proteins indicate a risk of hypersensitivity. Recent publications indicate that haplotype HLA B*5701 is a significant predictor of the risk of abacavir hypersensitivity,[10-15] meaning that such genetic analyses are useful for specifying which patients should not receive abacavir in the future.

The aim of the study is to identify, among patients likely to have a hypersensitivity reaction, those cases that developed a true, definitive hypersensitivity reaction to abacavir by determining the presence of allele HLA-B*5701.

Methods

We carried out a retrospective study with all adult patients infected with HIV-1 who received treatment with abacavir between January 2000 and December 2007 in Area 6 of the Valencia Health Care Agency (*Agencia Valenciana de Salud*). First, we analysed all patients whose treatment was discontinued due to a suspected hypersensitivity reaction to the drug. We considered documented cases of allergic reactions or the presence of 2 or more of the following symptoms: cutaneous eruptions, fever, gastrointestinal symptoms and lethargy or general malaise; respiratory symptoms; musculoskeletal symptoms; headache, paraesthesia; and oedema or hypotension.

We identified 39 patients with suspected HSR and classified them according to age, sex, co-infection with the hepatitis C virus (HCV), the antiretroviral combination, the initial and ending dates of abacavir treatment, whether or not the HLA-B*5701 allele was present, the CD8 and CD4 lymphocyte counts at the beginning of treatment, the HIV infection stage, and the number of treatments previous to starting abacavir.

With regard to the genetic markers, we carried out the analysis to screen for the HLA-B*5701 allele in the Valencian Community's transfusion centre laboratory; to identify HLA as class I, we used a combination of specific sequence oligonucleotide probes (SSOP) or a microlymphotoxicity assay, followed by a polymerase chain reaction with sequence-specific primers (PCR-SSP) to determine the presence of allele HLA-B*5701.

The results were processed using G-stat 2.0® statistical analysis software. The quantitative variables were studied according to the distribution and dispersion measurements (mean, maximum, and minimum) and qualitative measurements by absolute and relative frequency.

We used the χ^2 test to compare CD8 lymphocyte populations and sex with the presence of the HLA-B*5701 allele.

Results

A total of 323 patients received treatment with abacavir during the study period. Treatment was discontinued in 39 patients (12.1%) due to a probable hypersensitivity reaction. Table 1 shows the demographic variables, the HIV infection stage and the number of drug combinations in the treatment of the patient prior to including abacavir.

The most commonly used combination was abacavir-lamivudine-zidovudine, which was prescribed to 30.6% of the patients; a smaller proportion (16.7%) was treated with abacavir-lamivudine-tenofovir. Therteen point nine percent of patients were treated with abacavir-didanosine and a protease inhibitor (PI), normally atazanavir. None of the cases used a combination with efavirenz or nevirapine, since it could create confusion in the diagnosis; these drugs have a toxicity similar to that of abacavir (exanthema and gastrointestinal symptoms).

The patients were divided in seven subgroups (Table 2) according to the different signs and symptoms presented that were related to hypersensitivity reaction,[9] and the mean duration for the abacavir treatment was established.

Fifty-seven point four percent of patients developed exanthema as an HSR symptom. In Table 3, we see that the 7 groups of patients were classified by sex, being positive or negative for HLA-B*5701, the lymphocyte population and the viral load before starting treatment with abacavir.

Twenty-three point one percent of patients who developed a suspected HSR to abacavir were HLA-B*5701 positive: 2 women and 7 men between the ages of 30 and 50. One had not received antiretroviral drugs before starting treatment with abacavir. Only 2 cases presented a CD8 score <850 cells/mL and 6 patients had a CD4 lymphocyte count of 250-500 cells/mL at the moment when abacavir was introduced in their treatment.

No significant differences were found between sex and the presence of HLA-B*5701 (P=.5265).

Sixty-six point seven percent of the patients had a lymphocyte count of CD8+ >850 cells/mL. The distribution of these lymphocytes among patients whose genetic test was positive or negative showed no significant differences (P=.8601).

Discussion

In our study, 12.1% of patients treated with abacavir presented a suspected HSR value that was higher than in

Table 1 Characteristics of patients treated with abacavir who experienced hypersensitivity reaction (HSR)

	Patients with HSR
Males	27 (69.2%)
Females	12 (30.8%)
Age, y	43 (27-69)
Caucasian	38
Non-caucasian	1
HIV stage	
A	3 (7.7%)
B	15 (38.5%)
C	20 (51.3%)
Patients with no previous treatment	2 (5.1%)
Patients undergoing prophylaxis	1 (2.6%)
No. of antiretroviral combinations prior to abacavir treatment	3.3 (1-12)

HIV indicates human immunodeficiency virus.
Data expressed as n (%) or as a mean (interval).

Table 2 Classification of signs and symptoms of a hypersensitivity reaction (HSR)

HSR symptoms	Patients, n (%)	Time for symptoms to appear, mean (interval), d
Exanthema	16 (41)	10.4 (615)
Exanthema and fever	2 (5.1)	10.5 (10-11)
Cutaneous eruption, asthenia, hypotension	3 (7.7)	17.7 (6-32)
Musculoskeletal symptoms and cutaneous eruption	1 (2.6)	
Gastrointestinal symptoms (nausea, vomiting, or diarrhoea)	10 (25.6)	31 (4-65)
Gastrointestinal symptoms (nausea, vomiting, or diarrhoea) and/or fever, or hypotension	4 (10.3)	12 (8-14)
Headaches, paraesthesia, asthenia, oedema, and hypotension and/or dyspnoea	3 (7.7)	32 (6-80)

Table 3 Genetic, virological, and immunological differences between patient subgroups according to the signs and symptoms they presented

HSR	Sex		HLA-B*5701		Mean lymphocyte population		Viral load, copies/mL	
	Male	Female	Positive	Negative	$CD4^+$, cel/mL	$CD8^+$, cel/mL	Negative, %	>10 000, %
Exanthema	12	4	6	10	315	1206	69.2	30.8
Exanthema and fever	2	0	1	1	125	555	50	50
Cutaneous eruption, asthenia, hypotension	1	2	1	2	527	1170	100	0
Musculo-skeletal symptoms and cutaneous eruption	1	0	0	1	290	1300	100	0
Gastro-intestinal symptoms	9	1	0	10	648	932	83.3	16.7
Gastro-intestinal symptoms (nausea, vomiting, or diarrhoea) and/or fever, or hypotension	2	2	1	3	455	633	75	25
Headaches, paraesthesia, asthenia, oedema and hypotension and/or, dyspnoea	0	3	0	3	427	960	66.7	33.3

HSR indicates hypersensitivity reaction.

other studies, such as those by Hetherington et al[14] and Crutell et al,[7] in which the reaction took place in 4.3% and 5% of patients (interval, 0%-14%), respectively. If we examine only the clinical data, it shows that the results are heterogeneous, which is probably due to not being able to clearly identify cases of HSR caused by abacavir alone. The only way to clinically confirm the HSR is to reintroduce the drug and wait for the symptoms to appear, which is ethically unacceptable.

The symptoms related to an HSR to abacavir appear during the first few weeks and tend to grow worse with subsequent doses; discontinuing the drug results in the symptoms improving in 48-72 h.[16-18]

The most commonly found symptom was exanthema, in 56.4% of the patients, followed by gastrointestinal symptoms in 41%; only 7.7% presented a fever; and 5.1%, respiratory symptoms. Hypotension was also present in 7.7% of patients, but the HSR was not severe in any of these cases. All the analysed data are in line with the literature,[19] except that fever occurred in a smaller percentage of patients, which can probably be explained by the rapid withdrawal of the abacavir.

All of the symptoms occurred before completing 6 weeks of treatment. On average, they appeared at 22.2 days, which is a higher value than the bibliographic data that we found; this is probably due to the presence of misidentified HSR, since for HLA-B*5701 positive patients, the average was 7 days.

HLA-B*5701 screening was given to the 39 patients experiencing HSR since it is the most sensitive test to MHC alleles, as demonstrated by different studies.[11-14,20,21] In a recent study, PREDICT-1, the presence of HLA-B*5701 had a 100% positive prediction value for hypersensitivity reaction to abacavir and a negative prediction value of 97%.[10] That is, those authors showed that a specific human genetic

variation, known as HLA-B*5701, is related to susceptibility to abacavir hypersensitivity.[12]

Performing a screening test before beginning treatment will prevent diagnostic errors and enable us to interpret symptoms with more certainty.

Out of 323 patients being treated with abacavir, 9 presented the HLA-B*5701 allele, which represents 2.8% of all patients undergoing treatment. This value is within the data range obtained from different studies, which showed 1%-2% in the Mediterranean population.[10,22]

Of the 9 patients who tested positive, 8 presented a cutaneous eruption, and only 1 patient testing positive had gastrointestinal symptoms and fever.

The risk factors for developing HSR that we analysed include the use of efavirenz with nevirapine, which can lead to confusion in the diagnosis since their toxicity is similar to abacavir's.[23-25] The other antiretroviral drugs that form a part of the treatment can favour the appearance of other adverse effects not related to HSR that can cause confusion at the start of the treatment: gastrointestinal symptoms (diarrhoea, dyspepsia, nausea, vomiting) commonly appear in conjunction with the use of some protease inhibitors (ritonavir, lopinavir, etc).

A high CD8 lymphocyte count at the time of starting abacavir treatment is associated with HSR.[5,13,26-28] When the drug acts upon the cells presenting the MHC-I antigen, the response, which is specific to HLA-B*5701 triggers the blood delivery of cytokines by CD8 T-cells, causing HSR. The greater the CD8 activation, the higher the risk of developing HSR. The risk of HSR increases when the CD8 lymphocyte count is higher than 850 cells/mL. Of the 39 patients who were analysed, 66.7% had a CD8 count >850 cells/mL. Among the patients who tested positive for HLA-B*5701, only 1 had a CD8 lymphocyte score <850 cells/mL.

Patients who were positive in the genetic test were predominantly male (83.3%, similar to other published studies); women made up a minority of 16.7% in our study, and this percentage is comparable to that stated in the literature.[13,14]

Co-infection of HIV and HCV is a factor to be taken into account when evaluating the signs and symptoms of HSR, since a high percentage of HIV patients also have HCV, and the use of drugs for hepatitis C can lead to increased hepatotoxicity, as well as mistaking underlying liver disease symptoms for a hypersensitivity reaction.

It must be mentioned that out of the 39 patients that were analysed, one had not been previously treated; this patient was HIV negative and HLA-B*5701 positive, and underwent anti-HIV prophylaxis due to a biological accident. This confirms the relationship between HLA-B*5701 and HSR to abacavir, even in HIV-negative patients.

Conclusions

Use of the genetic test for HLA-B*5701 allows us to lower the risk of abacavir toxicity and eliminates the costs produced by treating HSR, which involve repeated visits to specialists, changes in treatment, the wasted abacavir and the addition of concomitant medication.[29] Performing this test has become a part of normal clinical practice, keeping in mind that only nine patients of the 39 for whom treatment was discontinued tested positive.

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