

Carbamazepine Hypersensitivity Syndrome Triggered by a Human Herpes Virus Reactivation in a Genetically Predisposed Patient

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Established Facts

- Carbamazepine may cause a severe or life-threatening hypersensitivity syndrome.
- Viral reactivation may trigger drug-induced hypersensitivity syndrome.
- Genetic variants of human leukocyte antigen and enzymes involved in the metabolism of carbamazepine are associated with the development of hypersensitivity but no unique determinant in the Caucasian population has been found.

Novel Insights

- This is the first report of an association between the human leukocyte antigen HLA*3101 and carbamazepine hypersensitivity in a subject of Caucasian ethnicity.
- The interaction of multiple genetic factors, and in particular human leukocyte antigen alleles and variants of the epoxide hydrolase gene, could be responsible for the triggering of the hypersensitivity reaction during treatment with carbamazepine.

Key Words

Carbamazepine • Hypersensitivity • Herpesviridae • Lymphocyte activation • Pharmacogenetics • Human leukocyte antigen • Epoxide hydrolases

Abstract

A case of severe hypersensitivity syndrome, triggered by carbamazepine in the presence of a concomitant active human herpes virus (HHV) 6 and 7 infection is described. To further understand the molecular mechanism of this adverse reac-

tion, analyses of the genetic variants of human leukocyte antigen (HLA) and of the epoxide hydrolase gene (*EPHX1*), previously associated with carbamazepine hypersensitivity, were performed. A lymphocyte transformation test (LTT) was conducted in order to detect drug-specific lymphocytes. In the hypersensitive patient, 2 genetic factors previously associated with intolerance to carbamazepine were detected: the allele HLA-A*3101 and homozygosity for the

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variant allele of SNP rs1051740 in *EPHX1*. Drug-specific lymphocytes could be detected by LTT when the HHV was active (positive PCR for viral DNA and increased anti-HHV 6 IgG titer), but not when it was no longer active. In conclusion, we document a case of severe carbamazepine hypersensitivity triggered by viral reactivation in a patient presenting the interaction of 2 unfavorable genetic factors.

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Introduction

The mechanism of carbamazepine hypersensitivity is not fully understood, as it results from a complex interaction between genetic and environmental factors. The clinical features of this reaction are suggestive of an immune-mediated response, as supported by the identification of drug-reactive T cells [1, 2], detected by the lymphocyte transformation test (LTT) test, which is based on the measurement of the proliferation of peripheral blood mononuclear cells (PBMC) in vitro in the presence of the drug [3]. Different factors have been shown to contribute to drug immune-mediated hypersensitivity: viral infection is one of the most well characterized and established environmental factors known to trigger these toxic reactions to carbamazepine and other chemicals [4, 5]. Among the genetic factors, in the Asian population the presence of a human leukocyte antigen (HLA)-B*1502 allele is strongly associated with the occurrence of severe and life-threatening adverse reactions during carbamazepine treatment [6]. In the Caucasian population, however, this genetic association has not been confirmed and, even if different genetic factors have been proposed, a unique genetic determinant of carbamazepine hypersensitivity has not been identified so far [7–9].

In this report, a case of severe hypersensitivity syndrome, triggered by carbamazepine in the presence of concomitant active human herpes virus (HHV) 6 and 7 is described. To further understand the molecular mechanism of this adverse reaction, analysis of genetic variants of HLA and of epoxide hydrolase gene (*EPHX1*), previously associated with carbamazepine hypersensitivity, was performed. An LTT was also carried out in order to detect drug-specific lymphocytes.

Case Report

A 12-year-old girl was evaluated by a neurologist for a first generalized epileptic seizure preceded by an absence. EEG, performed in an interictal period, showed some nonspecific abnor-

malities and a short phase, during sleep, of generalized spikes. A cerebral CT scan was normal and no therapy was instituted. Five months later a second episode of simple partial seizure occurred and treatment with sodium valproate was started. In the next month no further seizures occurred but, because of persistent EEG abnormalities, the dose of valproate was progressively reduced and carbamazepine was started.

One month after the beginning of carbamazepine, a 4-day episode of high fever, associated with cervical lymphadenopathy occurred, and the patient was treated with paracetamol and ibuprofen. After 3 days without fever, high temperature (above 39°C) appeared again along with an itchy erythematous rash diffuse throughout body. In the following days, the fever persisted and treatment with amoxicillin was started, sodium valproate was definitively stopped, while carbamazepine dose was reduced. The fever subsided for a few days, and then relapsed, together with the reappearance of a widespread itchy maculopapular erythema. The consultant neurologist decided to interrupt both carbamazepine and amoxicillin and the patient was hospitalized.

At admission the physical examination was remarkable for a distressed appearance, a cutaneous rash with mild face edema, and a moderate laterocervical lymphadenopathy.

Laboratory tests showed a normal hemochrome with moderate lymphopenia (800 cell/mm³), an increase in transaminase (AST 200 IU/l, ALT 181 IU/l) and of gamma-GT (116 IU/l) with negativity of inflammatory indexes.

Monotest and serology for Epstein-Barr virus, cytomegalovirus, toxoplasma and *Bartonella* were negative, as well as the immunologic assays for respiratory viruses (influenza, adenovirus, respiratory syncytial virus). A PCR based assay on blood tested positive for HHV 6 and 7. The titer of anti-HHV 6 IgG was determined to be more than 1:128; quantification of anti-HHV 7 IgG was not possible for technical reasons.

The history of recurrent fever together with skin rash, lymphadenopathy, liver involvement with normal acute phase reactants, moderate hypereosinophilia and negative Epstein-Barr virus after the starting of carbamazepine suggested an anticonvulsant hypersensitivity syndrome, possibly triggered by the reactivation of HHV.

An i.v. infusion of immunoglobulins (1 g/kg) was started. After 48 h, fever and rash persisted, with worsening of laboratory signs of hepatic involvement: increase of transaminases and appearance of coagulation alterations (INR 1.51, PT 57%, fibrinogen 268 mg/dl, antithrombin III 84%, D-dimer 3.5 ng/ml).

Intravenous methylprednisolone 1 mg/kg was started and continued for 3 days, passing then at the oral administration of the same drug. This allowed, in few days, a resolution of fever and of the skin rash together with a progressive normalization of laboratory parameters. Steroid therapy was then slowly tapered over 3 months.

To further understand the molecular mechanism of this adverse reaction, analysis of gene variants previously related to carbamazepine sensitivity was performed. In particular, the characterization of HLA was done, since some HLA alleles are strongly associated with the development of cutaneous adverse reactions induced by carbamazepine and by other medications [10]; moreover 2 common SNPs of *EPHX1*, previously associated with significant modifications in epoxide hydrolase activity, were genotyped: rs1051740 (T→C) in exon 3 and rs2234922 (A→G) in exon 4 (minor allele frequency in the Caucasian population 0.325 and

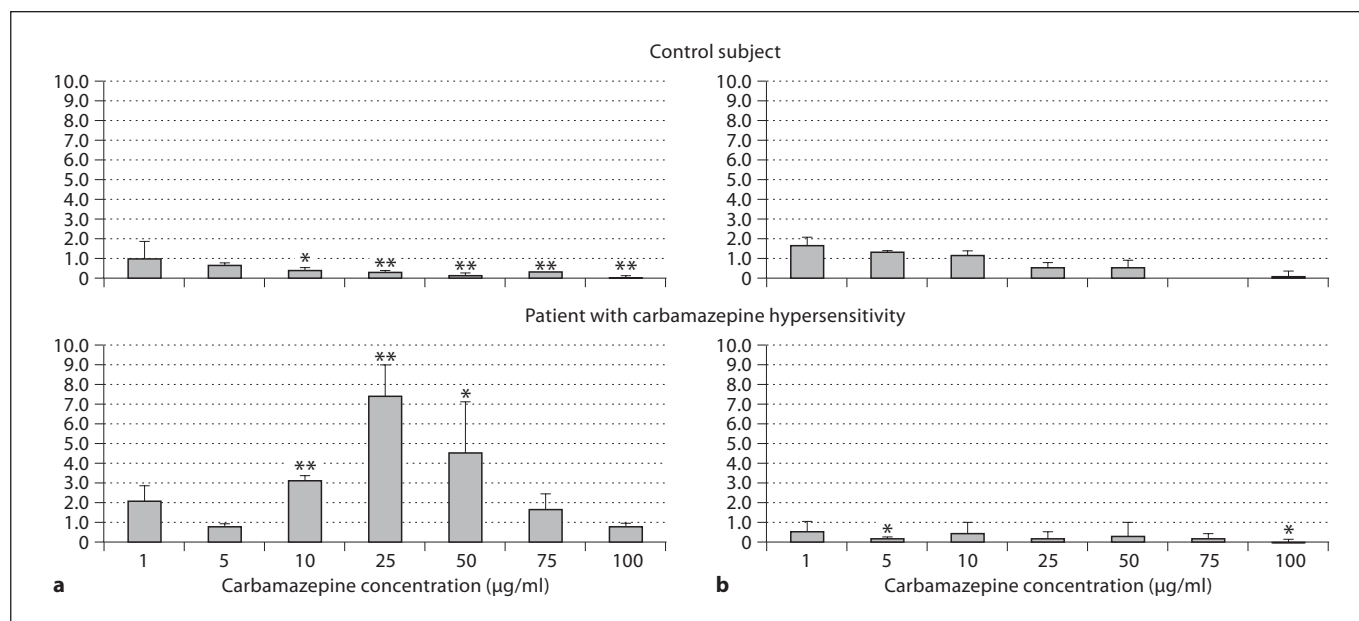


Fig. 1. Demonstration of dose-dependent carbamazepine-specific lymphocyte transformation; PBMC were collected from controls and from the patient when HHV 6/7 infection was active (**a**) and when it was not active anymore (**b**). PBMC from the hypersensitive patient were incubated with carbamazepine (at concentrations of 1–100 µg/ml) for 6 days, and proliferation was mea-

sured by [³H]-thymidine incorporation. Results are expressed as the mean stimulation indices $\pm$ SD of triplicate cultures. Statistical analysis was performed by comparing incubations in the presence of the drug with those in the absence of the drug. * $p < 0.05$; ** $p < 0.01$ (t test).

0.175, respectively [11]). Measurement of lymphocyte activation was done by means of lymphocyte transformation test, both during the period with active herpes virus (positive PCR and increased anti-HHV 6 IgG titer), and outside the infection period, one year later (negative PCR and no detectable anti-HHV 6 IgG antibodies), when the virus were not active anymore. Local ethical committee approval was received for these studies and appropriate informed consent was obtained.

HLA alleles were determined by sequence-specific oligonucleotide reverse lineblots as previously reported [12]; rs1051740 and rs2234922 polymorphisms of *EPHX1* where genotyped by two PCR-based assays, as described previously [13].

Proliferation of PBMC from patients and control volunteers was measured using LTT as described previously in detail [14]. Briefly, freshly isolated PBMC (1.5×10^5 ; total volume, 0.2 ml) were incubated with carbamazepine (1–100 µg) dissolved in dimethyl sulfoxide in 96-well tissue culture plates for 6 days (37°C; 5% CO₂). Proliferation was determined by the addition of [³H]-thymidine (0.5 µCi) for the final 16 h of the incubation period. Cells were harvested and [³H]-thymidine incorporation was measured as counts per minute (c.p.m.) on a β -counter. Proliferative responses were calculated as stimulation indices (c.p.m. in drug-treated cultures/c.p.m. in cultures with dimethyl sulfoxide alone).

Differences in the proliferation response between lymphocytes exposed to carbamazepine and controls were evaluated with Student's t test.

In the hypersensitive patients, the genotyping detected a *3101 allele for HLA-A, and, for *EPHX1*, a homozygous variant genotype for the SNP rs1051740, associated with a reduced activity of the enzyme. Genotyping of SNP rs2234922 showed a homozygous wild type genotype.

When the HHV was active, as demonstrated by a positive PCR for viral DNA and increased IgG titre, incubation of carbamazepine with PBMC from the hypersensitive patient, but not from the controls, caused a strong proliferative response, as measured by LTT: a statistically significant proliferation was seen with 10–50 µg ml⁻¹ of carbamazepine (fig. 1a).

One year later, when HHV was not active anymore, the same analysis was repeated twice but no significant proliferation was observed both for the hypersensitive patient and the negative control (fig. 1b).

Discussion

In the present report a case of severe hypersensitivity syndrome to the anticonvulsant carbamazepine, triggered by a reactivation of a viral infection in a genetically predisposed patient, is described. Viral infections have been shown to significantly increase the risk of drug induced hypersensitivity reactions [4], in particular those involving the skin. Indeed, the occurrence of skin reac-

tions to trimethoprim-sulfamethoxazole is substantially increased among patients with the human immunodeficiency virus infection [4]. Moreover, among patients receiving penicillins and other antimicrobial agents, the incidence of rashes is significantly higher in those with infectious mononucleosis [3]. For carbamazepine and other anticonvulsants, the association between reactivation of HHV, in particular HHV 6 and 7, and the development of hypersensitivity syndrome has been previously reported [15]; the reactivation of HHV has been associated even with hypersensitivity syndromes caused by other molecules like cyanamide [16] or mexiletine hydrochloride [17]. For all these drug-related hypersensitivity reactions, virus-induced immune modulation has been proposed as the triggering factor [5]. Modulation of the host immune response represents an important mechanism for the viruses to create an environment favorable for their growth and persistence, and this is particularly relevant for agents like HHV that typically persist for their host's entire lifetime [18]. Indeed, for HHV, different molecular mechanisms of modulation of the immune system have been described, such as infection of specific lymphocyte subpopulations and induction of inflammatory and immunosuppressive cytokines or chemokines [5, 19]. The precise molecular and cellular mechanism that allows the herpes virus to have this specific ability (as compared to other viruses) of triggering the skin reaction is not known and should be the object of further research.

Besides the viral infection, another nongenetic factor that could have been involved in the determination of the hypersensitivity episode described is the treatment with an association of valproic acid and carbamazepine, which are known to present a significant metabolic interaction [20].

The development of hypersensitivity also has a genetic component, since siblings of patients with anticonvulsant hypersensitivity are at increased risk of developing this syndrome [21]. Among the candidate genes involved in the metabolism of the drug, previous studies have considered the enzyme epoxide hydrolase, which is involved in carbamazepine metabolism. Carbamazepine is primarily metabolized by CYP3A4 to its reactive metabolite carbamazepine-10,11-epoxide, which is subsequently transformed by epoxide hydrolase to the inactive carbamazepine-10,11-diol [22]. Although variant alleles of the *EPHX1* gene have been reported to be more frequent in hypersensitive than in tolerant subjects, a unique genetic identifier could not be recognized [9]. The patient described in this study presented an *EPHX1* genotype as-

sociated with low enzymatic activity. This could have caused the accumulation of toxic epoxide metabolites during the treatment with carbamazepine, contributing to the triggering of the hypersensitivity reaction.

Among other candidate genes, HLA alleles have been related to different forms of carbamazepine cutaneous adverse reactions: in particular Stevens-Johnson syndrome and toxic epidermal necrolysis have been related to HLA-B*1502 allele [6, 12]. However, this association was shown in the Chinese population and was not confirmed in patients of Caucasian ethnicity, and for this reason it is considered race specific [7, 8, 23]. Not surprisingly, the patient described in this case did not present the HLA-B*1502 allele that is relatively rare in the Caucasian population (allele frequency 0.14 in Chinese and 0.058 in Europeans [24]). However, she presented the HLA-A*3101 allele that the previous report in the Chinese population had found to be associated with the development of maculopapular erythema from carbamazepine. The frequency of this allele is comparable among the 2 populations: 0.028 for the Chinese and 0.027 for the Europeans [24]. This is a first report of an association between this allele and carbamazepine hypersensitivity in a subject of Caucasian ethnicity.

In conclusion, we describe a case of severe carbamazepine hypersensitivity triggered by viral reactivation in a genetically predisposed patient. Further studies in larger populations of patients should be done to understand the interaction between nongenetic factors (viral infection, drug associations and others) and multiple genetic factors [25], which leads to the development of hypersensitivity reactions during anticonvulsant therapy, in order to develop rational strategies to predict and reduce the probability of these severe toxic events in patients needing these medications.

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