Genetic and Immune Predictors for Hypersensitivity Syndrome to Antiepileptic Drugs

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Abstract

Hypersensitivity syndrome reactions (HSR) to antiepileptic drugs (AED) are associated with severe clinical cutaneous adverse reactions (SCAR).

We aimed: to assess HSRs to AEDs using the *in vitro* lymphocyte toxicity assay (LTA) in patients who manifested HSRs clinically, to correlate LTA results with the clinical syndrome, to correlate LTA results with the human leukocyte antigen (HLA) allele B*1502 (HLA-B*1502) positivity in a Han Chinese-Canadian population, and to determine the cytokine network in this population.

HSR patients developed fever and cutaneous eruptions in the presence or absence of organ involvement within 8 weeks of exposure to carbamazepine (CBZ), phenytoin (PHY) or lamotrigine (LTG). Control patients received AEDs without presenting HSR. We investigated 10 CBZ-HSR (4 presented with Stevens-Johnson syndrome (SJS)), 24 CBZ-controls, 10 PHY-HSR (4 presented with drug-induced liver injury (DILI)), 24 PHY-controls, 6 LTG-HSR (1 SJS and 1 DILI) and 24 LTG-controls. There were 30 Han Chinese individuals (14 HSR patients and 16 controls) in our cohort. LTA toxicity greater than 12.5%±2.5% was considered positive. Differences among groups were determined by analysis of variance. In addition, we measured cytokine secretion in the patient sera between 1 month and 3 years after the event. All Han Chinese individuals and 30% of Caucasians were genotyped for HLA-B*1502.

A perfect correlation (r=0.92) was observed between positive LTA and clinical diagnosis of DILI and SJS/toxic epidermal necrolysis (TEN). HLA-B*1502 positivity in Han Chinese is a predictor of CBZ-HSR and PHY-HSR. HLA-B*1502-negative Han Chinese receiving only CBZ or a combination of CBZ-PHY tolerated the drug(s) clinically, presenting negative CBZ-LTA and PHY-LTA. However, 3 patients presenting negative CBZ-LTA and PHY-LTA, as well as negative HLA-B*1502, showed positive LTG-LTA (38%, 28% and 25%, respectively), implying that they should not be prescribed LTG. Three patients had LTA positive to both PHY and CBZ, and 3 others had LTA positive to both

PHY and LTG. Clinically, all six patients presented HSR to both drugs that they tested positive to (cross-reactivity). Patients were grouped based on the clinical presentation of their symptoms as only rash and fever or a triad that characterizes “true” HSR (rash, fever and DILI or SJS/TEN). Levels of pro-inflammatory cytokines were significantly higher in patient sera compared to control sera. More specifically, the highest levels of tumor necrosis factor (TNF)-α was measured in patients presenting “true” HSR, as were the apoptotic markers Fas, caspase 8 activity and M30. We concluded that he LTA is sensitive for DILI and SJS/TEN regardless of drug or ethnicity. HSR prediction will prevent AED-induced morbidity. In Han Chinese, HLA-B*1502 positivity is a predictor for CBZ-HSR and PHY-HSR. Its negativity does not predict a negative LTG-HSR. There is cross-reactivity between AEDs. Additionally, T-cell cytokines and chemokines control the pathogenesis of SJS/TEN and DILI, contributing to apoptotic processes in the liver and in the skin.

Abbreviations

ADR – adverse drug reaction

AED – antiepileptic drug

CBZ - carbamazepine

DIHS - drug-induced hypersensitivity syndrome

DILI – drug-induced liver injury

DRESS – drug rash with eosinophilia and systemic symptoms

Fas – CD95, APO-I

HLA – human leukocyte antigen

HSR - hypersensitivity syndrome reaction

IL - interleukin

LTA – lymphocyte toxicity assay

LTG - lamotrigine

M30 - mitochondrial marker for apoptosis (cytokeratine 18)

MHC – major histocompatibility complex

ox-CBZ - oxcarbazepine

PHY - phenytoin

RANTES - regulated upon activation normal T-cell expressed and secreted

SCAR - severe cutaneous adverse reactions

SJS - Stevens-Johnson syndrome

TEN - toxic epidermal necrolysis

Th – T helper response

TNF-α - tumor necrosis factor-α

Introduction

Increasing knowledge about the mechanisms involved in the development of seizures, as well as improved understanding of the cellular effects of antiepileptic drugs (AED), have resulted in links between demonstrated molecular actions of these drugs and the types of seizures against which they are effective. A number of AEDs have been synthesized, with the goal of adapting synaptic function in order to regulate seizure frequency or occurrence. “First generation” AEDs include carbamazepine (CBZ), phenytoin (PHY), phenobarbital and valporate, whereas felbamate, gabapentin, lamotrigine (LTG), topiramate, levitrcetam, oxcarbazepine (ox-CBZ) and zonisamide are classified as “second generation” AEDs. Aromatic AEDs include CBZ, PHY and phenobarbital.[1] CBZ and PHY are structurally related to one another, while LTG is not. PHY is para-hydroxylated by cytochrome p450s primarily to two enantiomers, and further metabolized to a catechol that spontaneously oxidizes to semiquinone and quinine species.[2-4] Major CBZ metabolism pathways include oxidation, hydration to 2- and 3-hydroxy-CBZ, which can be further oxidized to a catechol or an iminoquinone.[5,6] LTG is largely metabolized in the liver by glucuronic acid conjugation, producing a 2-N-glucuronide conjugate, which can be hydrolyzed to beta-glucuronidase.[7] Interactions between AEDs are important in examining drug function and metabolism. CBZ and PHY decrease the half-life of LTG in the body, while valporate increases it.[8]

Hypersensitivity reactions (HSR) are a common feature of anticonvulsants, being noted in 30% and 70% of patients with drug-induced liver injury (DILI) caused by CBZ and PHY, respectively.[9,10] To establish whether a drug is the cause of an immune-mediated reaction, alternative causes, latency of a reaction after drug intake, improvement after drug cessation, previous patient cases, and rechallenge have to be examined.[9]

CBZ, PHY and LTG have been associated with HSRs. LTG was reported to produce Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) and DILI.[11-13] Most HSR cases occur

during the first eight weeks of treatment.[14-17] HSRs refer to dose-independent, idiosyncratic, severe adverse drug reactions (ADR).[18,19] Clinically, a triad of fever, rash and organ manifestation defines a "true" HSR.[9,18,20,21]

Skin manifestations include exanthematous rash, blistering eruptions such as erythema multiforme, SJS and TEN.[16,22-25] SJS and TEN are severe cutaneous adverse reactions (SCAR).[26-34] Epidermal detachment below 10% is defined as SJS, whereas it is considered TEN above 30% [35,36]. Epidermal detachment between 10% and 30% defines transitional SJS/TEN.[37,38] The incidence of TEN is estimated between 1-2 cases/million people/year, with a mortality rate of 30%, whereas there are 2-7 cases/million people/year of SJS, with a mortality rate of 1-3%.[39,41] AEDs are among the leading causes of SCAR,[42-44] drug-induced hypersensitivity syndrome (DIHS)/drug rash with eosinophilia and systemic symptoms (DRESS)[42,45,46] and DILI.[47] Interestingly, while AEDs were the second leading cause of cutaneous ADRs, CBZ (10.3%) and PHY (9.6%) were the AEDs most often implicated, with CBZ the primary cause of SJS/TEN (24.0%) in Malaysia.[48]

Genetic markers such as the human leukocyte antigen (HLA) are useful in predicting an individual's predisposition AED-HSR. Ever since it was first linked with CBZ-SJS in Han Chinese,[49] HLA allele B*1502 (HLA-B*1502) has become the strongest HLA correlation among human diseases (98% negative predictive value, 92% sensitivity and 4.2%-19% false positivity).[50] This association holds true for certain ethnic groups only, in particular Han Chinese, Thai, Malay and to a lesser degree, Indians.[50,51]

HSR cases accompanied by hepatocytotoxicity are fewer than HSR cases with dermatologic presentations. DILI may lead to liver failure and transplant.[10,25]

Regardless of ethnicity, HSR predisposition can be predicted by the *in vitro* lymphocyte toxicity assay (LTA).[52,53] The mitochondrial enzyme succinate dehydrogenase-based LTA was validated versus clinical manifestations.[53] Neuman et al.[53]also validated the new colorimetric methodology

versus the previous LTA based on trypan blue uptake measured by microscopic counting of cells.[52] Reactive metabolites of drugs are generated using murine hepatic microsomes as a source of cytochrome p450s.[16,53,54] The LTA is based on the hypothesis that human lymphocytes *in vitro* mimic functional cells *in vivo*. Human lymphocytes from patients with a suspected HSR are used as surrogate target cells for safe *in vitro* rechallenge. Lymphocytes are suitable as they possess the patient's phenotype. They also contain detoxification enzymes such as epoxide hydroxylases and glutathione S transferases, while expressing phenotypic individual variability in these enzymes. The LTA was validated for AEDs such as CBZ, PHY, valproic acid, zonisomide and Phenobarbital.[25,53]

The HSR is a complex clinical manifestation, whose diagnosis rests mainly on clinical data. We demonstrated that predisposed individuals have lymphocytes that show cytotoxicity when exposed to the incriminated drug *in vitro*. The LTA remains a diagnostic tool applied successfully in AED-HSR in individuals with different ethnic backgrounds, which has never been directly compared to results of HLA genotyping.

Oxidative cell damage caused by the generation of reactive drug species may also cause or contribute to the release of cytokines that warn the immune system of cellular stress and damage.[16,55,56] Several signals may occur in a variety of settings, including infection and surgery, where they promote an immune response to eliminate these potentially dangerous calls.[57] T-cell-dependent immune responses encompass the type 1 T-helper response (Th1) associated with regulation of cell-mediated immune responses, or the type 2 T-helper response (Th2) which plays an important role in antibody or humoral immune responses.[57] Cytokines play a role in the immuno-pathological and molecular mechanisms of drug-induced HSR.[55] Alteration in the balance of Th1 cytokines, such as interleukins (IL) (IL-1, IL-2, IL-6, IL-18) and tumor necrosis factor (TNF)-α, and Th2 cytokines (IL-4, IL-10) are important factors in HSR. Pro-inflammatory

cytokines and chemokines act as signals for antigen-presenting cells, leading to necrosis, apoptosis and organ damage.[16]

This study aimed to: 1-assess AED-HSRs using the *in vitro* LTA in the ethnically-diverse Canadian population who manifested HSRs, including Han Chinese; 2-correlate LTA results to clinical symptoms; 3-correlate LTA results to HLA-B*1502 in Han Chinese-Canadians; and 4-determine the cytokine network.

Patients and Methods

PATIENTS

Patients with clinical HSR manifestation [rash, fever and organ (skin, liver) involvement] within 8 weeks of exposure to AEDs (CBZ, PHY and LTG) belonging to an epilepsy clinic (PH) or referred to a liver consult (LC) were included into the study. Experts have made the clinical diagnosis of type of seizure (PH), DILI (LC) and dermatological involvement (burn unit surgeons and dermatologist from the referral hospitals). Controls consisted of patients with epilepsy receiving AEDs without developing HSRs. The laboratory was blinded for clinical phenotypes of the participants.

We studied: 10 PHY-HSR (4 DILI), 24 PHY-controls, 10 CBZ-HSR (4 SJS), 24 CBZ-controls, 6 LTG-HSR (1 SJS and 1 DILI) and 24 LTG-controls. LTA toxicity higher than 12.5%±2.5% was considered positive.

The median time latency (interquartile range) between start of drug intake and index-day was less than 3 weeks [CBZ: 12 days (8-16), PHY: 12 days (10-18) and LTG: 9 days (4-20)].

There were 30 Han Chinese individuals (14 HSR patients and 16 controls). All Han Chinese individuals underwent HLA genotyping. The ethnical distribution of HSR patients and therapy-tolerant controls was 50% Han Chinese, 40% Caucasians, 8% Hispanic and 2% African-Americans.

The neurologist (PH) established the causality to a specific drug and the hepatologist (LC) was

consulted for cases presenting liver involvement. The clinical biochemist and pharmacology specialist (MN) was responsible for the study design, the laboratory diagnosis and the technical procedures, as well as writing the paper with the toxicology specialist (RN). Ethical approval for the study was obtained from the Scientific and Ethics Review Committees of the North York General Hospital, Toronto, Canada. All patients signed the informed consent form before participating in the study. The LTA was performed 2-3 years after the HSR in patients presenting at the clinic with a non-active adverse event. A single blood sample was drawn from each patient, which was used to extract the lymphocytes for the LTA and to obtain the sera for the cytokine analysis. Anonymous clinical description was matched to each sample.

CHEMICALS

Chemicals were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Microsomes were obtained from mice induced to a certain cytochrome p450 as previously described.[53] The microsomal solution was diluted in HEPES buffer. Gibco® (InVitrogen; Carlsbad, California, USA) is the manufacture of Dulbecco's modified eagle medium (α-MEM). Generic drugs (CBZ, LTG) were manufactured by Apotex Inc. (North York, Ontario, Canada, http://www.apotex.com/ca/en/search.asp).

PREPARATION OF THE LTA

Samples belonging to both sensitive and tolerant patients were incubated in the presence or absence of the microsomal fraction as previously described.[53] The parental drug was added at its therapeutic concentration.[53] The HLA polymorphism was determined using the method described by Kazeem et al.[58]

Enzyme-linked immunosorbent assay (ELISA) immunoassay kits for human IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, MCP1, regulated upon activation normal T-cell expressed and secreted (RANTES) and TNF-α (e-Bioscience, San Diego, CA, USA), as well as Fas, cleaved caspase 8, M30-

Apoptosense ELISA (Bender MedSystems, Vienna, Austria) were used for quantitative determination. We used standards and reference reagents available from Bender MedSystems (Vienna, Austria). Each specimen was analyzed in triplicate with 95% sensitivity and 90% specificity.

CALCULATIONS AND STATISTICS

LTA toxicity higher than 12.5%±2.5% was considered positive. These cut-off toxicity values were previously validated.[53] Results that do not exceed these parameters indicate a negative LTA. Differences among groups were determined by the use of confidence intervals (CI) and analysis of variance. The χ^2 test or Fisher's exact test was used to compare frequency data between groups. The Wilcoxon rank sum test was used to compare differences between groups. Binomial logistic regression was used to calculate the degree of correlation and predictability of the variables (clinical drug exposure results) to the LTA. The method used to estimate the model was the forward stepwise (likelihood ratio) method. The Student's 2-tailed *t-test* for independent samples was also performed against subgroups that were clinically tolerant or clinically hypersensitive within each drug treatment (CBZ, PHY and LTG) to illustrate whether toxicity results were distinct. Statistical analysis was performed using SPSS Version 12.0.1 (Chicago, Illinois, USA). We presented the results graphically using Microsoft Excel 2000 (Redmond, Washington, USA). Most of our patients took the therapeutics because of their chronic condition (i.e. epileptic seizures).

We also took into consideration all the non-drug risk factors such as chronic diseases (e.g. cancer, trauma) that required chronic therapeutic interventions in addition to AEDs. Also, we noted existing radiotherapy, recent viral disease or chronic viral infection.

Results

All patients with clinical manifestations to AEDs presented positive LTA to the incriminating drug. Controls tolerant to the drug presented negative LTA to the respective drugs. Figure 1 presents a graphical illustration of the LTA values of all patients tested. Patients were grouped based on the clinically presentation of their symptoms as follows: only rush and fever or a triad that characterizes “true” HSR (rash, fever and DILI or SJS/TEN), and the controls (individuals tolerating the drug). Result are given as mean percentage of toxicity and standard deviation (SD) of toxicity for each drug.

There were no statistically significant differences between the mean ages of sensitive versus clinically-tolerant patients (32.5±24.5 and 38.5±16.0) (p=0.256). The statistical analysis of patients with DILI or SJS/TEN indicated an LTA sensitivity of 92.9%. Patients presenting a marginal LTA clinically demonstrated rash and fever only. The negative predictive value was 98.9%. This suggests a strong true-positive rate. The specificity of the reaction was 99.1%, which nicely identifies patients without the disease. There were 3 patients who presented a positive LTA to CBZ and also illustrated a positive PHY-LTA. Also, 3 PHY-HSR patients showed a positive LTG-HSR. Clinically, these patients developed HSR with liver involvement after AED therapy.

A 76 year-old Caucasian male demonstrated cross-reactivity to CBZ and PHY. This individual presented SJS with liver involvement after PHY-CBZ therapeutic doses. His laboratory data showed high alanine aminotransferase (ALT) and elevated aspartate aminotransferase (AST) and bilirubin x 3 versus the normal. Two young Han Chinese patients showed SJS clinically to both CBZ and PHY with extremely elevated LTA values (53% CBZ-LTA and 28% PHY-LTA, and 38% CBZ-LTA and 26% PHY-LTA, respectively). The LTA for CBZ and PHY correlated with the clinical presentation and with HLA-B*1502 positivity. All 3 Han Chinese patients that had SJS also had positive HLA-B*1502. Our study confirms that CBZ-HSR can be predicted by the presence of HLA-B*1502, while an HLA-B*1502-negative status is protective against CBZ sensitivity in Han Chinese

patients. The LTA is predictive for both CBZ-HSR and PHY-HSR, and can be correlated with HLA-B*1502 positive results in Han Chinese individuals. The high cytotoxicity confirms the predictive value of the LTA in this population.

In the present work, we define the cytokine levels in the sera of controls tolerant to AED and HSR patients (Table 1). There is a clear partition between the levels of cytokines in controls, patients presenting only with rush, and patients presenting with the triad of rash, fever and organ involvement. Patients presenting with SJS/TEN and DILI show elevated levels of Fas and caspase 8, pointing to the apoptotic processes involved in the immunopathogenesis of severe adverse events such as DILI and SJS/TEN. There is a statistical difference between the levels of cytokines in control individuals and patients presenting adverse reactions and between patients presenting only with rash and patients presenting with DILI or SJS/TEN (Table 1).

Discussion

It is crucial to have a complete understanding of the biochemical processes that occur during HSR since it would allow for a better comprehension of any predisposing factors, potential cross-reactivity, and the required duration of treatment. Ultimately, this analysis would aid clinicians in their assessment and decisions regarding potential therapies or the possible switch of the present therapy to a more effective one. The LTA is an *in vitro* assay that allows assessment of potential HSRs to a drug metabolite. This test is based on mitochondria dysfunction and a lack of drug detoxification capabilities in people sensitive to certain drugs. Several surveillance strategies have evolved that limit mitochondrial damage and ensure cellular integrity. Intraorganellar proteases conduct protein quality control and exert regulatory functions, allowing mitochondria to protect against apoptosis. Cell death caused by the metabolite can be detected as mitochondrial damage upon exposure to reactive drug metabolites.[53] Mitochondrial

damage may only signify a fraction of the complications involved in HSR, but it is a critical one nonetheless. Defects in any of the five complexes in the respiratory chain would greatly add to mitochondrial toxicity.[57] The LTA is a marker of one such disorder, as it highlights genetic deficiencies in succinate dehydrogenase, a specific enzyme part of complex II in the mitochondrial respiratory chain. Since succinate dehydrogenase is specific to mitochondrial activity, its dysfunction would indicate cell death when the cell is subjected to stress.[53] We have already performed similar studies in individuals who presented HSR to AEDs.[16,53] The present study confirms that the LTA can predict an HSR to AEDs in an ethnically-specific population of Han Chinese individuals living in Canada, an environment different from that which their "mainland" ancestors were exposed to. There is no overlap in the patients studied in the prior work and the patients studied in the present work. The present work validates the usefulness of this diagnostic and predictive assay to be utilized in possible HSR reaction to AEDs, mainly in individuals of Asian descent living in Canada, while the main population studied in our previous studies was Caucasian. In the current study, AED samples were segregated into tolerant and hypersensitive groups.

Cross-reactivity to anticonvulsants was observed clinically for a number of decades between CBZ, PHY and valproic acid.[33,59-64] In the present work, we observed cross-reactivity between LTG and CBZ. The phenomenon may be explained by the ability of T-cells to recognize the major histocompatibility complex (MHC) of a drug-MHC complex. We demonstrated that the LTA is able to indicate a possible clinical AEDs cross-reactivity.

Early diagnosis of an HSR is important in improving the quality of life in patients. The high frequency of rashes and a lack of efficacy are the main reasons for medication substitution in anti-epilepsy therapy. To avoid possible HSRs, testing is strongly advised. The LTA allows neurologists a routine analysis to predict and prevent an adverse reaction. The test may

eventually help elucidate the mechanism of cytotoxicity caused by reactive metabolites, and the patterns of inheritance of defects in detoxification pathways.

Our group analyzed the composition of circulating cytokines and their cellular expression in lymphocytes, using markers of cell activation such as the Fas/Fas ligand system.[16] Our results confirmed that the apoptotic pathway is significantly activated in SJS/TEN.[55] Fujita et al. described the role of inflammatory cytokines IL-1 and IL-5, and chemokines CCL5, CCL17 and CXCL10 in cutaneous immune inflammation.[65] In our previous work,[55] we demonstrated the immunochemical presence of apoptosis and necrosis markers in the skin biopsy of a patient with TEN, as well as the clear distribution of pro-inflammatory cytokines in the sera of patients with ibuprofen-induced SJS/TEN.

The present work confirms that an HSR is a clinical reaction with genetic (HLA) and immune (cytokines) markers significantly different in susceptible versus tolerant individuals. Also, levels of cytokines differ between patients presenting only rash and patients presenting the triad of rash, fever and organ involvement (Table 1). Patients presenting with SJS/TEN or DILI show elevated levels of Fas and caspase 8, pointing to the apoptotic processes involved in the immunopathogenesis of severe adverse events such as DILI and SJS/TEN (Table 1). Cytokines released by the inflammatory process have the potential to alter oxidative drug metabolism, thereby increasing the production and toxicity of reactive drug metabolites therefore leading to toxic events. Pro-inflammatory cytokines expressed as a result of T-cell activation may represent a danger signal for HSR, as demonstrated by our findings. However, excluding neutrophils, these cells are also antigen-presenting cells, and are thus capable of biotransformation, conjugation and immune cell stimulation. In the present work, there is a positive correlation between LTG-DILI/SJS cases and the serum level of chemo-attractant substances such as chemokines (IL-8, RANTES and monocyte chemo-attractant protein). Similar results have been shown in our

previous work.[57] Pro-inflammatory cytokines such as IL-1α and TNF-α are molecules that stimulate the synthesis of acute-phase proteins.[66] IL-18 is pro-inflammatory at a very early step in the immune response. IL-6 stimulates most acute-phase proteins, while IL-10 is a prototype anti-inflammatory cytokine that regulates B-cell function.[16] Moreover, Fas was shown to be dysregulated in SJS/TEN.[55,67] T-cell memory is responsible for the recognition of "stress" signals represented by the exposure of lymphocytes to the insulting drug. We demonstrated a clear link between genetic factors such as HLA and immune responses to a specific injury. The more severe the injury, the higher the level of inflammatory responses and apoptosis were.

The present work also takes into consideration the possibility that patients that are prone to HSR may develop cross-reactivity due to a structural similarity between CBZ and PHY, but also to an agent with a different structure and chemical properties, LTG. For example, one Han Chinese individual presented an HSR to both CBZ and PHY. The drugs were substituted with phenobarbital, which was well tolerated. Due to clinically-insufficient efficacy of phenobarbital, the patient was prescribed LTG. After two weeks of LTG therapy, the patient presented with LTG-HSR. This patient presented a highly-positive LTA to CBZ, PHY and LTG, but negative to phenobarbital. We have demonstrated that the LTA is a powerful tool that can predict an HSR and cross-reactivity to AEDs regardless of the patient's ethnic background. Should an LTG-LTA have been performed prior to administering this agent, the LTG-HSR could have been avoided.

Our results show that LTG sensitivity is not significantly correlated to the presence of HLA-B*1502. One HLA-B*1502-negative Han Chinese patient with negative CBZ-LTA and without clinical CBZ-HSR was in concordance with the previous observations showing that a negative HLA-B*1502 status permits safe CBZ use in Han Chinese individuals.[68] However, he presented a high LTG-LTA (38%), showing that he is sensitive to LTG and should not take it. The LTA can thus be used to prevent an HSR. Another Han Chinese patient developed clinical SJS after a therapy

with oxy-CBZ, CBZ and PHY. The patient presented a positive HLA-B*1502. LTA-PHY was positive (20%) and LTA-CBZ was highly positive (53%). However, the same patient showed only 7% toxicity to LTG-LTA. Therefore, if the LTA would be performed before prescribing the drugs, the SJS could be avoided by administering the safe LTG. This rationale has an important clinical implication, demonstrating that HLA-B*1502 is not predictive of LTG-HSR, and will permit safe LTG administration. These cases represent a confirmation that a positive HLA-B*1502 is a predictor for CBZ-HSR and PHY-HSR in Han Chinese population, but not for LTG-HSR.

Chung et al. found that HLA-B*1502 was present in 100% of CBZ-SJS patients, 3% of CBZ-tolerant patients, and 8.6% of the general population among Han Chinese in Taiwan.[49] The presence of this allele was predictive in 93.6% of cases, while its absence had a negative-prediction value of 100%.[49] Similar findings emerged from other Han Chinese populations.[68-71] A significant association between CBZ-SJS/TEN and HLA-B*1502 was also found in Thai,[72,73] Malay[74] and, to a lesser degree, Indian patients.[75] In a European study, the only HLA-B*1502-positive CBZ-SJS patients were of East Asian descent.[76] No association was found in Japanese, Korean or Caucasian individuals. HLA-A*3101 was recently associated with CBZ-HSR, CBZ-maculopapular exanthema and CBZ-SJS/TEN in North European Caucasians,[77] while HLA-B*1511 is associated with CBZ-SJS/TEN,[78] and HLA-A*3101 with CBZ-cutaneous ADRs in Japanese.[79]

HLA-B*1502 was associated with CBZ-induced SJS/TEN, but not with CBZ-induced mild maculopapular eruptions in central China.[80] HLA-B*1502 was a risk factor for ox-CBZ-induced mild maculopapular eruptions,[81] ox-CBZ- SJS/TEN and PHY-SJS/TEN.[68] PHY-HSR was further associated with of HLA-B*1301, Cw*0801 and HLA-DRB1*1602 in a Chinese population.[68]

The high incidence of HLA-B*1502 among Asian individuals (10-15%) correlates with a high incidence of SJS/TEN in this population (17 to 25 in every 10000 individuals in Taiwan and Thailand), compared to its low incidence among Caucasians (1% to 2%), which correlates with a

low incidence in this population (1 to 6 in every 10000 individuals).[49,50,82]

No association was found between LTG-SJS/TEN and HLA-B*1502 in Chinese,[68] or European patients.[58] In our study, we observed that negative HLA-B*1502 does not predict negative LTG-HSR. We conclude that HLA-B*1502-negative status is not protective against LTG-HSR. For HLA-B*1502-negative patients wishing to take LTG, an LTA is highly advised in order to ensure tolerance towards this anticonvulsant. The main limitation of our study is the relatively small number of Han Chinese patients presenting AED-HSR who participated in the study.

Conclusion

Current results demonstrate that the LTA is a good predictor tool for possible HSRs in individuals with epilepsy. HLA-B*1502 positivity represent a biomarker for possible CBZ-HSR and PHY-HSR in specific Asian populations, especially Han Chinese. More importantly, this work shows for the first time that HLA-B*1502 negativity does not prevent LTG-HSR in Han Chinese. The study also reinforces that immune mediated components, such as T-cells and their cytokines and chemokines products, can exacerbate cellular responses and create complex pathways that lead to cell apoptosis and consequently to a variety of clinical manifestations. Additionally, it has been confirmed that the LTA is a valuable method for predicting and confirming HSR to a specific drug. We have proposed that the LTA could be used as a screen tool for those considering taking or changing AEDs, as means to avoid an HSR. Our study emphasizes the necessity to wisely monitor the patients clinically and by laboratory investigations when prescribing AEDs, in order to avoiding AED-HSRs. Personalized medicine and assessing individual risk to adverse drug reaction is important for physicians and patients, since alternatives for the drugs involved may be needed for future safe treatment.

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Table 1

Cytokines (mean ± SD)	Control 24 x 3 = 72	Patients 10 PHY + 10 CBZ + 6 LTG = 26	
		Rash + Fever 6 PHY + 6 CBZ + 4 LTG = 16	SJS/TEN (4 CBZ + 1 LTG) + DILI (4 PHY + 1 LTG) = 10
Cytokines (mean ± SD)	Control 24 x 3 = 72	Patients 10 PHY + 10 CBZ + 6 LTG = 26	
		Rash + Fever 6 PHY + 6 CBZ + 4 LTG = 16	SJS/TEN (4 CBZ + 1 LTG) + DILI (4 PHY + 1 LTG) = 10
IL-1 pg/mL	45±15	60±25	220±20^
IL-2 pg/mL	35±15	70±15	260±10^
IL-4 pg/mL	64±10	46±14	22±4#
IL-5 pg/mL	25±15	166±16*	350±15^
IL-6 pg/mL	38±10	36±10	49±6
IL-8 pg/mL	69±10	108±15	195±15^
IL-10 pg/mL	30±5	40±12	65±25
MCP 1 pg/mL	30±2.5	36±4	320±22^
RANTES pg/mL	50±20	105±15*	150±15^
TNF-α pg/mL	50±10	130±15*	170±10^
Fas ng/mL	2.0±1.0	12±4*	20.0±5.0^
M30 (%)	18±5	28±15	40.0±10.0^
Caspase 8 IU/mL	5.0±1.0	18±8	25.0±15.0^
Caspase 3 IU/mL	4.0±1.0	18±12	35.0±10.0^

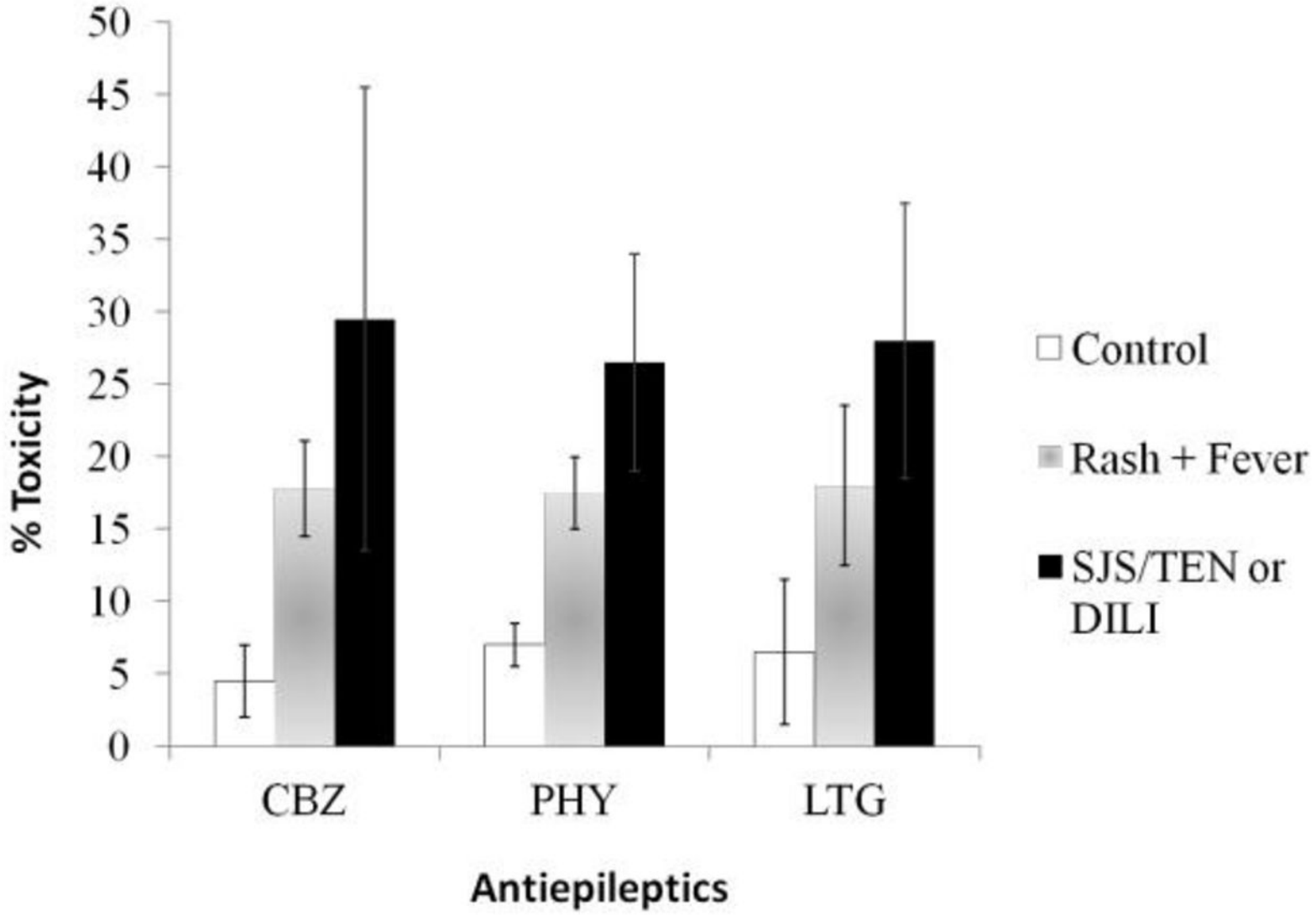
^p<0.001 higher vs. control

*p<0.05 higher vs. control

#p<0.05 lower vs. control

Figure 1 LTA values for all patients

Patients were grouped based on the clinically presentation of their symptoms as controls tolerating the drug (white), HSR patients presenting only rash and fever (grey) or patients presenting a triad of rash, fever and SJS/TEN or DILI that characterizes “true” HSR (black). The LTA value is given as mean percentage of toxicity and standard deviation (SD) in lymphocyte toxicity for each drug.


50
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% Toxicity
CBZ
PHY
LTG
Antiepileptics
Control
Rash + Fever
SJS/TEN or DILI