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Predictive Value of the Lymphocyte Toxicity Assay in the Diagnosis of Drug Hypersensitivity Syndrome

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Abstract

Background: Drug hypersensitivity syndrome (DHS) is a rare but potentially fatal adverse drug reaction that develops in susceptible patients following exposure to certain drugs. Because of the variable clinical picture of DHS and its resemblance to other diseases, the diagnosis of DHS is challenging. The lymphocyte toxicity assay (LTA) is an *in vitro* test that has been used in the diagnosis of DHS. However, its predictive values are still controversial because of the lack of a 'gold standard' test to measure it against.

Objectives: To determine the sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of the LTA in the diagnosis of DHS due to different classes of drugs, based on systemic re-exposure as a gold standard, and to evaluate the current clinical utility of the LTA in clinical practice.

Methods: Potential participants were identified from their medical records and contacted to obtain their consent to participate in the study. One hundred forty-seven patients were recruited and interviewed by telephone to identify events of re-exposure and their consequences. These data were used to determine true positive, false positive, true negative, and false negative results of the test, which were then used to estimate the predictive value of the test.

Results: We identified 26 re-exposure events in 22 patients: 4 were true positives, 17 were true negatives, 1 was a false positive, and 4 were false negatives, as determined by systemic re-exposure. Although the number of identified re-exposures limited the ability to calculate the predictive values, our data provide an estimate of the clinical value of the test for the diagnosis of DHS. The data also highlight the effect of the type of drug involved in the reaction on the predictive value of the test.

Conclusion: The LTA is potentially a valuable diagnostic tool for DHS; however, its sensitivity, specificity, NPV, and PPV seem to vary according to the drug involved in the reaction.

Introduction

The term 'adverse drug reaction' (ADR) is defined as any noxious and unintended response to a drug that occurs at a dose normally used in humans for prophylaxis, diagnosis, or therapy.[1] Most ADRs are predictable, dose-dependent, and related to the pharmacologic action of the drug (type A), accounting for 75–80% of all reported ADRs. The remaining 20–25% are defined as type B ADRs, which are unpredictable, are unrelated to the known primary pharmacologic action of the drug, and do not have a clear dose-dependency.[2,3] As they are patient-specific and unpredictable, such reactions are also known as 'idiosyncratic drug reactions' (IDRs). Typically, IDRs occur in small fraction of patients at the normal therapeutic dose.[4] Unfortunately, the underlying mechanisms of IDRs are poorly understood, although a large portion of IDRs are believed to be immune mediated, at least in part, as a delay in onset and rapid occurrence upon rechallenge are typical.[5] Drug hypersensitivity syndrome (DHS) is considered to be one type of immune-mediated IDR, characterized by a constellation of signs and symptoms that develop in susceptible patients, following exposure to certain drugs.[6] DHS is most commonly associated with the use of NSAIDs, aromatic anticonvulsants, antibiotics, and sulfonamide antimicrobials.[7]

It has always been a challenge to establish the diagnosis of DHS, because of its variable clinical picture and the lack of a safe and reliable diagnostic test. Another challenge is the fact that, with the exception of a fixed drug eruption, DHS can be mimicked by other non-drug-related illnesses (e.g. bacterial or viral infections).[4,8,9] In addition to the importance of identifying susceptible patients to establish safe clinical practice, it is essential not to falsely label a patient as hypersensitive to a drug that could be otherwise therapeutically useful. This is especially important in cases where alternative therapy is more risky or less effective (e.g. first-line therapy for epilepsy, or allopurinol for gout). Thus there is a need to develop a safe and reliable test to confirm the diagnosis and identify the culprit drug.

We have recently performed systematic reviews to evaluate the usefulness of different *in vivo* and *in vitro* diagnostic tests in the diagnosis of DHS due to aromatic anticonvulsant drugs.[10,11] These tests included the skin patch test, the lymphocyte transformation test, and the lymphocyte toxicity assay (LTA). It was quite evident that the predictive values (both negative predictive values [NPVs] and positive predictive values [PPVs]) of these tests were not clear, that their usefulness in clinical practice is controversial, and that more research is needed to prove or disprove their clinical usefulness. A systemic rechallenge or provocation test is considered the 'gold standard' in the diagnosis of DHS. However, this approach is not always ethically plausible, because of potentially severe reactions that may develop.[8,12,13]

The LTA was first developed by Spielberg et al.[14-17] in the 1980s to investigate patient susceptibility to DHS. The test is based on the concept that DHS develops as a result of an imbalance between activation (toxication) and detoxication of drugs *in vivo* (the reactive metabolite hypothesis).[18] This hypothesis has provided a partial explanation for the observation that some patients appear to be genetically predisposed to develop such reactions and others are not. Genetic polymorphism in enzymes involved in both activation and detoxication of drugs has been demonstrated in DHS patients and healthy controls.[19-21] The test includes isolation of peripheral blood lymphocytes (peripheral blood monocytes [PBMCs]) from blood samples from patients and healthy controls, and incubation of these lymphocytes with the culprit drug for 2 hours at 37°C in the presence of a metabolic activation system (e.g. rat liver microsomes) in suitable media. After an overnight recovery period at 37°C, cell viability is then measured using different methods (the trypan blue exclusion method or the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method).[14,22] It has been demonstrated that cells from hypersensitive patients are more susceptible to cell death induced by incubation with the culprit drug than cells from healthy controls. This observation has resulted in the clinical application of the LTA for the diagnosis of DHS and identification of the culprit agent, usually among a group of suspected drugs.[17,23,24]

The main obstacle that hinders accurate determination of the predictive values of this test is lack of a safe gold standard test to measure it against. As a result, there are presently only sparse clinical data to confirm the LTA after re-exposure.[10] In an attempt to close this gap, we performed this cohort study in a group of patients who had been tested using the LTA for susceptibility to DHS. We aimed to identify individuals who might have deliberately or inadvertently been exposed to the culprit drug(s) after having been tested. Our objective was to use these cases of re-exposure to calculate the NPV and PPV of the test.

Methods

Patient Recruitment and Data Collection

One hundred forty-seven patients were included in the study. These patients had developed hypersensitivity reactions to different drugs and were tested using the LTA between 1991 and 2008 in two drug safety clinics (the Sunnybrook Health Sciences Centre, Toronto, ON, Canada; and the Children's Hospital of Western Ontario, London, ON, Canada). The drugs that were assessed included aromatic anticonvulsants (phenytoin, carbamazepine, phenobarbitone, and lamotrigine), valproic acid, sulfonamides (sulfamethoxazole, sulfasalazine, and sulfapyrazine), β-lactam antibacterials (amoxicillin and cefaclor), macrolide antibacterials (erythromycin and clarithromycin), tetracycline, codeine, and accutane. Subjects were identified from their medical records at the two locations. The inclusion criteria were as follows: (i) the patient developed DHS as evaluated by the clinic specialist; (ii) the patient was tested for susceptibility to DHS, using the LTA; and (iii) consent was obtained from the patient or a parent (in the case of children) to participate in the study. Patients were excluded from the study if their files did not contain sufficient information (e.g. contact details or LTA results) or if they did not consent.

Ethical approval was obtained from the Research Ethics Boards of the Sunnybrook Health Sciences Centre and the University of Western Ontario, and information letters were sent to each potential participant prior to contacting them. Verbal consent was obtained from all participants or their parents (in the case of children) prior to inclusion in the study.

Preparation of Rat Liver Microsomes

Adult Sprague-Dawley rats (mean weight 200 g) were sacrificed by decapitation, and their livers were quickly isolated

under aseptic conditions and washed with ice-cold 0.5 M potassium phosphate homogenization buffer. The livers were then diced with scissors and homogenized in 3 volumes of homogenization buffer using a drill-powered Potter-Elvehjem homogenizer. The homogenates were then centrifuged at $9000 \times g$ for 30 minutes at 4°C. The supernatants were then centrifuged at $100\,000 \times g$ for 1 hour at 4°C. The pellets were then resuspended in homogenization buffer and stored at –80°C until they were used. The microsomal protein content was determined by the method of Lowry et al.[25]

Blood Collection and Isolation of Peripheral Blood Monocytes

Blood samples from patients and healthy volunteers were obtained by venepuncture, collected into heparinized syringes, and separated immediately. To isolate lymphocytes, samples were diluted 1 : 1 with phosphate-buffered saline [PBS] (NaCl 137 mM, KCl 2.7 mM, NaH_2PO_4 10 mM, KH_2PO_4 2 mM; pH 7.4) and layered on a Ficoll-Paque™ density gradient. Gradients were then spun at $500 \times g$ for 20 minutes, the aqueous Ficoll interface layer was collected and washed twice with PBS, and the cell density was adjusted to 1×10^6 cells per mL in 4-(2-hydroxymethyl)-1-piperazineethanesulfonic acid (HEPES)-buffered saline (HEPES 15 mM, NaCl 125 mM, KCl 6 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 1.0 mM, $CaCl_2$ 1.0 mM, glucose 10 mM; pH 7.4). At this stage, cell viability was determined using the trypan blue exclusion method and was always greater than 95%. All steps were performed in aseptic conditions in a laminar flow hood.

The Lymphocyte Toxicity Assay

The LTA was performed as previously described.[14] Briefly, 100 μL of the lymphocyte (PBMC) suspension at a density of 1×10^6 cell/mL was placed in each well of 96-well, flat-bottom plates. The cells were incubated with either the drug (at concentrations ranging between 6.5 and 500 μM for aromatic anticonvulsants and beta-lactam antibacterials) or its reactive metabolite (at concentrations ranging from 50 to 800 μM for sulfonamides) for 2 hours in a humidified atmosphere at 37°C and 5% CO_2 partial pressure. In experiments where the parent drug was used, the metabolic activation system (rat liver microsomes, prepared as described above) was included in the incubation media. Microsomal protein was added at a concentration of 0.25 mg/mL, followed by addition of the NADPH-generating system (nicotinamide adenosine dinucleotide phosphate [NADP] 0.6 mM, glucose-6-phosphate 2.4 mM, glucose-6-phosphate dehydrogenase 2 U/mL). The plates were then spun down at $500 \times g$ for 15 minutes, and the media were replaced with fresh RPMI 1640 media containing 10% fetal bovine serum and penicillin/streptomycin cocktail. The cells were then left to recover for 18 hours in a humidified atmosphere at 37°C and 5% CO_2 partial pressure. At this point, the plates were spun down and resuspended in 100 μL of HEPES buffer. Twenty-five microliters of MTT at 5 mg/mL was added to each well and incubated at 37°C for 4 hours, and the reactions were stopped by adding 100 μL of stop solution (NN-dimethylformamide [DMF] 50%; sodium dodecyl sulphate [SDS] 20%) and allowed to set overnight at room temperature, protected from light. The absorbance was measured at 590 nm by a molecular device spectrophotometer (Beckman, Palo Alto, CA, USA). A standard curve was generated by seeding the cells at 0%, 25%, 50%, 75%, and 100% of the cell concentration in the corresponding buffer in quadruplicate. The data were analyzed using Softmax™ Molecular Device Group Analytical software (Version 2.35), and statistical graphs were created using Microsoft Excel™ 2007 software. A cut-off value of a 15% increase in cell death over baseline (control) was used to determine positive tests. The tests were performed either in the lab of M.J. Rieder at the University of Western Ontario or in the Drug Safety Clinic at the Sunnybrook Health Sciences Centre.

Follow-Up Interviews

Information obtained from the patients' medical charts included patient age, sex, drugs tested, signs and symptoms of the reaction, and LTA results. The participants were then asked to answer a standard questionnaire through telephone interviews. The questionnaire was designed to identify whether the patient was exposed to the drug after being tested using the LTA and to find out the clinical response to the re-exposure. Patients were also asked whether they had experienced allergic reactions to any other drugs or had any family history of such reactions. The data that were obtained were analyzed to estimate the predictive value of the test in the diagnosis of DHS.

Results

One hundred forty-seven patients were recruited, consisting of 81 females (55.1%) and 66 males (44.9%), ranging between 3 and 89 years of age (mean 30.1 years). The LTA was performed on these patients for reactions to the following drugs/drug classes: aromatic anticonvulsants (n = 124), sulfonamides (n = 72), β-lactam antibacterials (n = 76), macrolide antibacterials (n = 10), ciprofloxacin (n = 3), valproic acid (n = 3), accutane (n = 2),

tetracycline (n = 1), and codeine (n = 1). The LTA results were found to be negative in 179 tests (61.3%) and positive in 113 tests (38.7%). The majority of patients avoided any use of the suspected drugs after the first event, regardless of the LTA test result. Of the 147 patients who were studied, a subgroup of 22 patients (15.0%) had been exposed to a tested drug. In total, of 26 events of re-exposure, 21 took place after a negative LTA and 5 after a positive LTA. The demographic characteristics of the patients included in the study are listed in table I.

The clinical data collected after the first events varied among patients, including skin rashes (erythmatous or blistering), fever, edema, gastrointestinal tract symptoms, hepatitis, lung diseases, lymphadenopathy, and hematologic abnormalities.

Of the 26 cases of re-exposure that occurred in 22 patients, 4 were true positive, 17 were true negative, 1 was false positive, and 4 were false negative, as determined by systemic re-exposure (table II). Based on the limited number of re-exposures that were identified, the NPV of the LTA in the diagnosis of DHS varied according to the drug that was tested. Of the 13 cases of re-exposure to β-lactam antibacterials, 2 were true positives, 8 were true negatives, and 3 were false negatives. This suggests low sensitivity and high specificity of the test in the diagnosis of DHS due to these drugs. On the other hand, the sensitivity of the LTA in the diagnosis of DHS due to sulfonamides may have been higher, as no false negative results were identified among the 7 cases of re-exposure. Five re-exposures to aromatic anticonvulsants revealed four true negatives and one false negative. The overall sensitivity and specificity of the LTA in the diagnosis of DHS, based on our 22 cases, were estimated to be 40% and 90%, respectively.

Table I. Characteristics of patients with drug hypersensitivity syndrome (DHS)

Characteristic	Value
Sex (female/male; n [%])	81/66 [55/45]
Age (mean; y [range])	30.1 [3–89]
Type of reaction (n [%])	
skin rash only	45 [31]
systemic involvement	83 [56]
none[a]	19 [13]
Drug tested (n [%])[b]	
sulfonamides	72 [25]
β-lactam antibiotics	76 [26]
aromatic anticonvulsants	124 [43]
others	20 [7]

a Patients whose clinical data were unavailable.

b Patients may have been tested for more than one drug.

Table II. Characteristics of systemically re-exposed patients, their lymphocyte toxicity assay (LTA) results, and the results of re-exposure

Patient no.	Age (y)	Sex	Drug tested	LTA result	Re-exposure
1	15	Male	Amoxicillin	–	+
2	9	Male	Amoxicillin	–	+
3	12	Female	Sulfamethoxazole	–	–
4	18	Female	Sulfamethoxazole	–	–
5	66	Female	Sulfamethoxazole	–	–
6	63	Female	Amoxicillin	–	–
7	23	Female	Cefaclor	+	+
8	62	Female	Sulfamethoxazole	+	+
9	10	Female	Amoxicillin	–	–
10	25	Male	Sulfamethoxazole	+	–
			Sulfamethoxazole	–	–
11	17	Male	Cefaclor	–	–
12	17	Male	Cefaclor	–	–
13	13	Female	Amoxicillin	–	–
14	22	Male	Accutane	+	+
15	9	Male	Cefaclor	+	+
16	12	Male	Amoxicillin	–	+
17	52	Female	Phenobarbital	–	–
18	14	Male	Phenytoin	–	–
19	82	Female	Carbamazepine	–	–
20	14	Male	Phenobarbital	–	+
			Lamotrigine	–	–
21	8	Female	Amoxicillin	–	–
22	11	Female	Sulfamethoxazole	–	–
			Cefaclor	–	–
			Amoxicillin	–	–

+ indicates a positive test result; – indicates a negative test result.

Discussion

DHS is a rare disorder with high rates of morbidity and mortality. It represents a challenging clinical problem and increases the cost of patient care. Although early diagnosis of DHS is essential for patient safety, that is not always achievable, because of the lack of a safe and reliable diagnostic test. The LTA has been a promising diagnostic tool for such reactions, but its clinical value has not been determined. To our knowledge, this is the first study to use a 'gold standard' test (systemic re-exposure) to validate the LTA. All previous attempts were based on correlation of suspected clinical manifestations and the history of exposure with the LTA test. Clinical manifestations of DHS are quite variable, and it is often difficult to

associate a typical clinical picture with this disease.[26] Furthermore, a temporal relationship between administration of the suspected drug and development of the disorder is known to be of poor predictive value, especially in cases where multiple drugs are used.[14]

Previous studies have attempted to measure the diagnostic value of the test. Naranjo et al.[27] evaluated the performance of the LTA in the diagnosis of drug hypersensitivity reactions, using a Bayesian statistical instrument to determine causality in 51 patients with suspected drug reactions. They estimated that the LTA had a specificity of 75% and a sensitivity of 99% in their studied cases. However, their study still lacked a 'gold standard' measure such as systemic rechallenge. In another study to validate the LTA in the diagnosis of DHS, Neuman et al.[22] studied 86 patients with suspected reactions to sulfamethoxazole, 62 patients with suspected reactions to anticonvulsants, and 26 healthy volunteers. They estimated the NPV of the test to be 64% and the PPV to be 90%, with sensitivity and specificity of 98% and 89%, respectively. However, no re-exposure data were available. In cases of reactions to sulfonamides, we found that the test had a high NPV. This is possibly due in part to the simplified procedure used to test sulfamethoxazole, which does not include *in vitro* metabolic activation. The toxic metabolite of sulfa drugs has been identified and synthesized, and was found to be adequately stable to be used experimentally for *in vitro* testing.[28] This step provided a more simplified and apparently more sensitive test. The PPV of LTA in highly suspected cases of DHS due to sulfamethoxazole has been estimated previously to be between 80% and 90%.[29,30] However, in these studies, the parent drug – not the metabolite – was used in the *in vitro* toxicity testing. In our study, however, the NPV for drugs such as β-lactam antibiotics (e.g. amoxicillin and cefaclor) was low, which may be explained by the more complicated pathophysiology of hapten-mediated reactions caused by these drugs.[31] Previous studies have suggested that the performance of LTA in cases of hypersensitivity to cefaclor depends largely on the type of reaction.[32] Because of the retrospective nature of our study, it was not possible to re-evaluate the patients clinically or to classify them according to the type of reaction. Inclusion of cases in our study with mixed types of reaction could have accounted for the relatively low NPV of LTA in β-lactam antibacterial cases.

The low sensitivity of the LTA test in β-lactam antibacterial cases is probably due to the complicated procedure of the test when using the metabolic activation system for drugs that need to be metabolized to one or more reactive (cytotoxic) metabolites. This is further supported by the good sensitivity of the test among sulfamethoxazole-induced cases.

Considering our incomplete understanding of the pathophysiology underlying DHS, it is difficult to speculate on how patient cells would react to the suspected drug *in vitro*. Drugs such as β-lactam antibacterials are known to cause different types of reactions, and they are classical hapten-forming agents.[33] Different molecular mechanisms and requirements are thought to underlie each type of these reactions (i.e. cell-mediated vs IgE-mediated), and some of them may not be detectable by the *in vitro* LTA test.[6] Furthermore, the variable clinical picture of the DHS may lead to difficulty in identifying affected patients. Among our cases, 30.6% developed a type of skin rash only, and 56.5% developed other systemic signs, which included fever, hepatitis, respiratory diseases, hematologic abnormalities, gastroenteritis, facial edema, anaphylaxis, angioedema, lymphadenopathy, and serum sickness-like reactions.

It is well established that 50% of all drugs in common use may cause some type of skin rash and that, in addition to fixed drug eruption, exanthematous (morbilliform, maculopapular, or scarlatiniform) and urticarial rashes are the more common cutaneous adverse reaction.[34] Exanthematous and pustular eruptions are more commonly associated with DHS than urticarial rashes, which are usually caused by immediate type (type I) hypersensitivity reactions. The pathophysiologic mechanisms of both types of reactions are thought to be different.[6] Taken together, one may expect that the LTA can have different predictive values in cases of both types of reactions; however, positive LTA tests were observed in this cohort in cases with all types of cutaneous reactions (data not shown).

The LTA test detects the vulnerability of patient cells to the reactive metabolite(s) of the drug. According to the reactive metabolite hypothesis, drug activation is a prerequisite for DHS to develop, and this could be a common step in the cascade of events that lead to the development of different types of reactions. This is one of the potential advantages of the LTA; it detects the genetic susceptibility of the patient to develop DHS, thus the test can also be used for patient screening prior to prescribing a potentially causative drug in high-risk populations.

Although the number of re-exposed cases was relatively low for performing comparative statistics, our data provided a fair evaluation of the test performance in the diagnosis of DHS based on the definite power of systemic re-exposure. There is no doubt that better-controlled prospective studies, using more defined inclusion criteria, are required to determine the true clinical value of this test; however, there are several technical and ethical obstacles that must be overcome before such studies can be conducted.

We have demonstrated another critical aspect of the LTA that deserves discussion. From our data, it is evident that in most cases (88.3%), patients avoided reusing the suspected drug

(probably in accordance with their doctors' advice) despite reassuring negative results. This finding strongly suggests a situation of 'mistrust' of the clinical value of the test, which is a direct result of the lack of evidence-based data on its predictive value. It is unlikely that a diagnostic test with an unknown predictive value will contribute to better clinical practice.

Conclusion

The LTA can be a valuable diagnostic tool for patient susceptibility to DHS, although the currently used procedure is quite complex and requires specialized experience. Further research may yield a more simplified test that is adaptable for wide clinical use. Such research is driven by the urgent need for a safe and reliable diagnostic test for DHS.

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References

1. World Health Organization. International drug monitoring: the role of national centres. World Health Organ Tech Rep Ser 1972; 498: 1-25
2. Rawlins M, Thompson J. Mechanisms of adverse drug reactions. In: Davies D, editor. Textbook of adverse drug reactions. Oxford: Oxford University Press, 1991: 18-45
3. Riedl MA, Casillas AM. Adverse drug reactions: types and treatment options. Am Fam Physician 2003 Nov 1; 68 (9): 1781-90
4. Uetrecht J. Idiosyncratic drug reactions: current understanding. Annu Rev Pharmacol Toxicol 2007; 47: 513-39
5. Uetrecht J. Immune-mediated adverse drug reactions. Chem Res Toxicol 2009 Jan; 22 (1): 24-34
6. Rieder MJ. Immune mediation of hypersensitivity adverse drug reactions: implications for therapy. Expert Opin Drug Saf 2009 May; 8 (3): 331-43
7. Demoly P, Viola M, Rebelo Gomes E, et al. Epidemiology and causes of drug hypersensitivity. In: Pichler W, editor. Drug hypersensitivity. Basel: Karger, 2007: 2-17
8. Bircheler AJ. Approach to the patient with a drug hypersensitivity reaction – clinical perspectives. In: Pichler W, editor. Drug hypersensitivity. Basel: Karger, 2007: 352-65
9. Nagao-Dias AT, Teixeira FM, Coelho HL. Diagnosing immune-mediated reactions to drugs. Allergol Immunopathol (Madr) 2009 Mar-Apr; 37 (2): 98-104
10. Elzagallaai AA, Knowles SR, Rieder MJ, et al. In vitro tests for the diagnosis of anticonvulsant hypersensitivity syndrome (AHS): a systematic review. Mol Diagn Ther 2009; 13 (5): 313-30
11. Elzagallaai AA, Knowles SR, Rieder MJ, et al. Patch testing for the diagnosis of anticonvulsant hypersensitivity syndrome: a systematic review. Drug Saf 2009; 32 (5): 391-408
12. Aberer W, Bircher A, Romano A, et al. Drug provocation testing in the diagnosis of drug hypersensitivity reactions: general considerations. Allergy 2003 Sep; 58 (9): 854-63
13. Lammintausta K, Kortekangas-Savolainen O. Oral challenge in patients with suspected cutaneous adverse drug reactions: findings in 784 patients during a 25-year-period. Acta Derm Venereol 2005; 85 (6): 491-6
14. Shear NH, Spielberg SP. Anticonvulsant hypersensitivity syndrome: in vitro assessment of risk. J Clin Invest 1988 Dec; 82 (6): 1826-32
15. Spielberg SP. Acetaminophen toxicity in human lymphocytes in vitro. J Pharmacol Exp Ther 1980 May; 213 (2): 395-8
16. Spielberg SP. In vitro assessment of pharmacogenetic susceptibility to toxic drug metabolites in humans. Fed Proc 1984 May 15; 43 (8): 2308-13
17. Spielberg SP, Gordon GB, Blake DA, et al. Predisposition to phenytoin hepatotoxicity assessed in vitro. N Engl J Med 1981 Sep 24; 305 (13): 722-7
18. Knowles SR, Uetrecht J, Shear NH. Idiosyncratic drug reactions: the reactive metabolite syndromes. Lancet 2000 Nov 4; 356 (9241): 1587-91
19. Lee AY, Kim MJ, Chey WY, et al. Genetic polymorphism of cytochrome P450 2C9 in diphenylhydantoin-induced cutaneous adverse drug reactions. Eur J Clin Pharmacol 2004 May; 60 (3): 155-9
20. Ingelman-Sundberg M. Genetic susceptibility to adverse effects of drugs and environmental toxicants: the role of the CYP family of enzymes. Mutat Res 2001 Oct 1; 482 (1-2): 11-9
21. Gaedigk A, Spielberg SP, Grant DM. Characterization of the microsomal epoxide hydrolase gene in patients with anticonvulsant adverse drug reactions. Pharmacogenetics 1994 Jun; 4 (3): 142-53
22. Neuman MG, Malkiewicz IM, Shear NH. A novel lymphocyte toxicity assay to assess drug hypersensitivity syndromes. Clin Biochem 2000 Oct; 33 (7): 517-24
23. Neuman MG, Shear NH, Malkiewicz IM, et al. Predicting possible zonisamide hypersensitivity syndrome. Exp Dermatol 2008 Dec; 17 (12): 1045-51
24. Rieder MJ, Uetrecht J, Shear NH, et al. Diagnosis of sulfonamide hypersensitivity reactions by in-vitro 'rechallenge' with hydroxylamine metabolites. Ann Intern Med 1989 Feb 15; 110 (4): 286-9
25. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. J Biol Chem 1951 Nov; 193 (1): 265-75
26. Peyriere H, Dereure O, Breton H, et al. Variability in the clinical pattern of cutaneous side-effects of drugs with systemic symptoms: does a DRESS syndrome really exist? Br J Dermatol 2006 Aug; 155 (2): 422-8
27. Naranjo CA, Kwok MC, Lanctot KL, et al. Enhanced differential diagnosis of anticonvulsant hypersensitivity reactions by an integrated Bayesian and biochemical approach. Clin Pharmacol Ther 1994 Nov; 56 (5): 564-75
28. Rieder MJ, Uetrecht J, Shear NH, et al. Synthesis and in vitro toxicity of hydroxylamine metabolites of sulfonamides. J Pharmacol Exp Ther 1988 Feb; 244 (2): 724-8
29. Neuman MG, Shear NH, Malkiewicz IM, et al. Immunopathogenesis of hypersensitivity syndrome reactions to sulfonamides. Transl Res 2007 May; 149 (5): 243-53
30. Neuman MG, Malkiewicz IM, Phillips EJ, et al. Monitoring adverse drug reactions to sulfonamide antibiotics in human immunodeficiency virus-infected individuals. Ther Drug Monit 2002 Dec; 24 (6): 728-36
31. Levine BB. Immunologic mechanisms of penicillin allergy: a haptenic model system for the study of allergic diseases of man. N Engl J Med 1966 Nov 17; 275 (20): 1115-25
32. Kearns GL, Wheeler JG, Childress SH, et al. Serum sickness-like reactions to cefaclor: role of hepatic metabolism and individual susceptibility. J Pediatr 1994 Nov; 125 (5 Pt 1): 805-11
33. Rodriguez-Pena R, Antunez C, Martin E, et al. Allergic reactions to beta-lactams. Expert Opin Drug Saf 2006 Jan; 5 (1): 31-48
34. Alanko K, Stubb S, Kauppinen K. Cutaneous drug reactions: clinical types and causative agents. A five-year survey of in-patients (1981-1985). Acta Derm Venereol 1989; 69 (3): 223-6

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