In vitro tests to identification of culprit drugs for severe cutaneous drug reactions

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ABSTRACT:

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Drug hypersensitivity reactions (DHRs) are considered as an important public health problem because they can lead to life-threatening conditions. The DHRs occur in certain people and are often not predictable. The most commons of severe DHRs are anaphylaxis and severe cutaneous adverse drug reactions (SCARs) containing acute generalized exanthematous pustulosis, drug reaction with eosinophilia and systemic symptoms syndrome, and Stevens-Johnson syndrome/toxic epidermal necrolysis. Although clinical evaluation with causality assessment methods is a non-invasive method to define suspicious drug, the majority of assessment grading falls into probable or possible. Without dedicate investigation, it is difficult to identify culprit drug. As severe DHRs are life-threatening conditions, drug provocation test has not been recommended and other *in vivo* skin tests have to be performed cautiously. It has been recommended that *in vitro* tests (if available) could be performed prior to any *in vivo* tests. Therefore, *in vitro* diagnostic tests could be an alternative for SCARs diagnosis with culprit drug identification. As the most common of

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severe DHRs are immediate and delayed type hypersensitivity reactions, there are many tests approached to identify causative agents for both reactions such as ELISA, ELISpot, basophil activation test (BAT) and lymphocyte transformation test (LTT). Nevertheless, BAT and LTT are functional *in vitro* tests serve as more reliable among *in vitro* tests for immediate and delayed type hypersensitivity reactions. Both BAT and LTT has been performed and broadly available in many countries, including Thailand. They have been promising tests that contribute to management of SCARs in clinical practice.

Key words: Drug hypersensitivity; severe cutaneous adverse drug reactions, basophil activation test, lymphocyte transformation test

Introduction

Classification of drug hypersensitivity reactions

Based on immunological mechanisms, Gell and Coombs classified drug hypersensitivity (allergic) reactions (DHRs) into 4 types as: type I; IgE mediated (or immediate type) reactions, type II; antibody-mediated cytotoxic reaction, type III; immune-complex mediated reaction and type IV; T-cell mediated (or delayed type) reaction[1]. Type IV has been recently classified in 4 subtypes as IVa, IVb, IVc and IVd, according to cytokine patterns and the preferential activation of different immunocytes[2]. However, common skin manifestations of DHRs are two types of hypersensitivity reactions: immediate type and delayed type hypersensitivity reactions, depending on the onset of symptoms after drug administration.

Immediate type hypersensitivity reaction (type I hypersensitivity reaction) can present as mild symptoms (urticaria, angioedema, conjunctivitis, rhinitis) or severe reaction such as anaphylaxis[3]. The most common presentations include urticaria/angioedema. Antibiotics (especially beta-lactam) and radiocontrast media (RCM) are the most common cause of fatal anaphylaxis[4].

Delayed type hypersensitivity reaction (type IV hypersensitivity reaction) is also common in DHRs. The severity of reaction is vary, ranging from mild maculopapular exanthema (MPE) to generalized cutaneous lesions with other organ involvements[3,5,6]. Recent immunological knowledge indicated that T cells play an essential role on delayed DHR, which cytokine profiles produced by various subsets of T cells are used to be further sub-categorized as following[7].

Type IVa: Th1 cytokines, especially IFN-γ, involve in type IVa reaction. These cytokines secreted by drug-specific T cells to activate macrophages leading to production of pro-inflammatory cytokines and booster CD8+ T-cell responses. These cytotoxic CD8+ T cells in inflammatory milieu can cause skin damage

manifested as MPE. The mild reaction usually occur a few days after the drug administration[3].

Type IVb: Type IVb reaction corresponds to Th2 immune responses with IL-4, IL-5 and IL-13 cytokine production. The characteristic eosinophilic inflammation due to high IL-5 production can be found in many drug hypersensitivity reactions, ranging from mild MPE to severe fatal reactions[2,8]. Drug reaction with eosinophilia and systemic symptoms syndrome (DRESS) is representative of type IVb reaction and present with morbilliform rash, fever, eosinophilia, atypical lymphocytosis and liver involvement[2]. Anti-epileptic drugs (carbamazepine, phenytoin, phenobarbital), allopurinol, salfasalazine, and some anti-retrovirals (nevirapine) are major culprit drugs associated with DRESS[6,9].

Type IVc: Cytotoxic T cells participate in type IVc reaction by killing keratinocytes through a perforin/granzyme B, granulysin and/or FasL-dependent manner[10]. Not only CD8+ T cells function as cytotoxic effectors cells but also CD4+ T cells can mediate cytotoxicity, albeit to a lower degree than CD8 cells. Apart from bullous skin diseases, these cytotoxic T cells also play a role in drug-induced mucous membrane erosion, hepatitis and nephritis[6]. In severe bullous skin reactions such as Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN), keratinocytes are killed by activated CD8+ T cells, and a massive accumulation of CD8+ T cells are found in the blister fluid of SJS/TEN[11,12]. Sulfonamide antibiotics, anticonvulsants, allopurinol, nevirapine, and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed drugs associated with SJS/TEN[6,9,13].

Type IVd: Sterile neutrophilic inflammation driven by T-cells is characteristic of type IVd and manifested as acute generalized exanthematous pustulosis (AGEP). It is an acute widespread edematous erythema followed by sterile small non-follicular, intraepidermal or subcorneal pustules (<5 mm) on an erythematous background. The cutaneous sterile neutrophilic inflammation is due to recruitment of neutrophils to skin by effect of CXCL8 secreted from activated T cells[14]. Approximately 90% of AGEP is associated with drug administration[15]. Antibacterial drugs, especially β-lactam antibiotics, and carbamazepine anticonvulsant are common etiologies[6,9].

SCARs assessment

Diagnosis of SCARs is important to safe life of patients, which the diagnosis is based on the history and clinical manifestations. However, identification of causative substance or culprit drug is also particularly important in severe cases, which the drug provocation test should be avoided[15,16]. To clarify culprit drug, assessment of clinical data, history of suspicious drug exposure, by many non-invasive assessment algorithms has

been proposed[17,18]. Unfortunately, the majority of assessment outcome is usually dropped into probable or possible, which could not be efficient for physician to ensure whether the patient is susceptible to the suspicious drug. Therefore, many diagnostic tests (both *in vivo* and *in vitro*) have been launched. Among *in vivo* tests, drug provocation test (DPT) is a gold standard to diagnose DHRs with culprit drug determination[19]. However, DPT is contraindicated in uncontrollable life-threatening DHRs[3]. Therefore, *in vitro* tests could be an alternative method served for culprit drug identification of SCARs diagnosis. In addition, *in vitro* tests are not invasive procedures (except for venous puncture) and the safest methods as compared to *in vivo* tests, especially DPT. *In vitro* tests were developed corresponding to mechanism of immediate and delayed type hypersensitivity reactions.

Culprit drug determination

***In vitro* tests for immediate DHR**

There have been many approaches to develop *in vitro* test mimicking to mechanism of immediate hypersensitivity reaction. Drug-specific serum IgE test has been proposed[20,21]. However, this test is not functional test and cannot simulate to outcome of immune cell response to cause hypersensitivity as well as there are many limitations of this test, which is out of scope of this review. Apart from drug-specific serum IgE test, basophil activation test (BAT) was proposed as it could simulate immediate hypersensitivity[20,22]. Principle of BAT was described elsewhere, in brief, the BAT is a functional assay used to detect change of activation markers (commonly CD63 and/or CD203c) on cell surface, when basophils get activated by IgE-crosslinkage with antigens[23]. Both CD63 and CD203c are representative markers of degranulation and determined by flow cytometry analysis. The BAT used to identify culprit drugs in immediate drug hypersensitivity was summarized in Table 1.

Table 1 Summary of recent reports on utilization of BAT to identify culprit drug for immediate DHR diagnosis

Drugs	Markers	Sensitivity (%)	Specificity (%)	References
Beta-lactam	CD63	49-55	91-100	(24, 25)
Quinolones	CD63, CD203c	71	100	(26, 27)
NMBAs	CD63	80-100	96-100	(28, 29)
RCM	CD63	46-63	89-100	(30, 31)
NSAIDs	CD63	37-61	90-91	(32, 33)

NMBAs; neuromuscular blocking agents, RCM; radiocontrast media, NSAIDs; non-steroidal ant-inflammatory drugs

As shown in Table 1, BAT was used to studies in immediate drug hypersensitivity diagnosis for beta-lactams, quinolones, neuromuscular blocking agents (NMBAs), RCM, and NSAIDs. Sensitivity of BAT is modest, however, its specificity is excellent. Therefore, the power of negative predictive value, corresponding to excellent specificity, is useful for clinician to manage further investigation to obtain definite diagnosis[34]. In case of life-threatening, BAT (if available) is recommended to perform prior to *in vivo* tests including skin test[35]. Some issues of BAT interpretation have to be concerned as limitation; inter-laboratory standardization is not consensus, blood sample in resolution phase could give false negative, nonallergic cause of DHR (especially in case of NSAIDs) is not ruled out, and uninterpretable cases of nonresponses have to be aware[36].

Table 2 Summary of recent reports and our unpublished data in Thai people on utilization of LTT to identify culprit drug for delayed SCARs diagnosis

Drugs	Clinical manifestation	Previous reports			Our unpublished data	
		Sensitivity (%)	Specificity (%)	References	Sensitivity (%)	Specificity (%)
Beta-lactam	AGEP	100	100	(40)	33	100
	DRESS	73	100	(41)	50	100
	SJS/TEN	66-100	100	(42, 43)	66	100
Antibiotics (Quinolone, co-trimoxazole, macrolide, anti-tuberculosis)	LABD	ND	ND	-	100 (vancomycin)	ND
	AGEP	100	ND	(44)	100	ND
	DRESS	83-100	33-100	(41)	83	100
	SJS/TEN	33	100	(40, 45, 46)	50	ND
Anti-epileptic drugs	DRESS	56-100	91-100	(41, 47, 48)	ND	ND
	SJS/TEN	40-64	94-96	(47, 48)	ND	ND
Allopurinol/ Oxpurinol	DRESS	16-33	98	(46, 49)	20	ND
	SJS/TEN	50	ND	(46)	66	ND

AGEP; acute generalized exanthematous pustulosis, DRESS; drug reaction with eosinophilia and systemic symptoms, SJS/TEN; Stevens-Johnson syndrome/ toxic epidermal necrolysis, LABD; linear IgA bullous dermatosis, ND; not determined

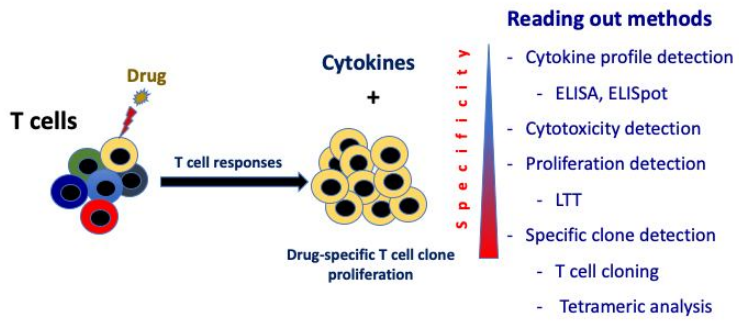

Figure 1

***In vitro* tests for delayed DHR**

As T cells play an important role in mechanism of delayed DHR[37]. Hence, many modality of readout systems, such as cytokine measurement, T cell proliferation and T cell clone detection, have been proposed to seek for drug-specific T cells to drugs elucidate the cause of SCARs as illustrated in Figure 1[38]. Cytokine detection methods, such as ELISA and ELISpot, could be generally available setting, whereas many points have to be concerned. Cytokine selection suitable for AGEP, DRESS, SJS/TEN, cut-off points for each cytokine, standardization between laboratories await for consensus. These concerned points attribute to low specificity and unreliability of these tests. Look at high specificity test, seeking for drug-specific T cell clone could be a goal, however, time and cost of setting are not possible to for routine service. Lymphocyte transformation test (LTT) developed base on detection of T cell proliferation is possible and has been employed among various aforementioned readout systems[16,39]. Principle of LTT is based on existence of drug-specific memory T-cell precursors able to proliferate upon re-stimulating with recalled culprit drugs[16]. Once activated, proliferative responses of drug-specific lymphocytes response could be measured by consumption of labelled nucleoside used for cell division. Level of the cell proliferation is ratio between lymphocytes stimulated with suspicious drugs compared with spontaneous background of lymphocyte proliferation, its ratio is called stimulation index (SI). The LTT used to identify culprit drugs for each type of delayed SCARs was summarized in Table2.

As shown in Table 2, beta-lactams, antibiotics (quinolones, co-trimoxazole, macrolide), anti-epileptic drugs and allopurinol were common drugs identified by LTT as culprit drugs causing

delayed SCARs. Even sensitivity of LTT is various depending on drugs and type of delayed SCARs, its sensitivity, in general, was higher when AGEP and DRESS samples subjected to perform with LTT as compared to sensitivity of LTT assayed with SJS/TEN samples. Importantly, specificity of LTT is excellent. As LTT has been recommended to perform with blood samples in recovery phase of delayed DHR cases because LTT performed with acute phase samples provided lower sensitivity as compared to its performance with recovery phase samples as well as false positive results were ever demonstrated in LTT assayed with acute phase samples[16,50]. LTT, therefore, is not test of choice to identify culprit drug in acute phase of SCARs. LTT cannot be performed to identify certain drugs, such as immunosuppressant drugs and chemotherapy as well as LTT could give false positive in case of testing some drugs (vancomycin, NSAIDs, and RCM)[35].

Requirement for *in vitro* tests

To identify culprit drug for definite SCARs diagnosis, drug provocation test are not recommended to perform and *in vivo* tests have to be performed with well-trained allergists/dermatologists with sufficient of medical equipment for resuscitation in proper place. European Network for Drug Allergy/European Academy of Allergy and Clinical Immunology released position publication suggested that *in vitro* tests, if available, could be first line to identify culprit drug prior to *in vivo* tests[35]. However, it not possible to request *in vitro* test all the time, many conditions have to be concerned before requesting *in vitro* tests as summarized in Table 3.

Additionally, how to prepare patients is also important step to avoid false result from *in vitro* tests. In general, all patients have no fever or evidence of systemic inflammatory responses as well as pancytopenia has to be omitted for *in vitro* tests. As BAT is set to determine activation of basophils, which antihistamine drugs do not affect on BAT, therefore, patients can continue to use antihistamine drugs[51-53]. In case of systemic corticosteroid and immunosuppressant drugs, these drugs have to be discontinued prior to *in vitro* test at least 5 half-lives. Nevertheless, there was an evidence that LTT could be performed in some decent conditions if blood samples drawn from patients who take < 0.2 mg/kg/day of prednisolone or who take methotrexate/ azathioprine without lymphopenia[16]. In case of urgency, there was a report to demonstrate that IFN-γ ELISpot could be utilized to identify culprit drug in delayed type DHR, however, clinical manifestation and data have to be correlated[54].

Table 3 Conditions for BAT and LTT

Conditions	BAT	LTT	
Proper time to request	After recent reaction 1-2 weeks (out of refractory period)	AGEP, SJS/TEN	After recovery 1 month
		DRESS	After recovery 3 months
Shorter time improper to request	Immediately until 1-2 week after recent reaction	AGEP, SJS/TEN	Within 1 month after recovery
		DRESS	Within 3 months after recovery
Longer time improper to request	More than 1 year after recent reaction	More than 2-3 years after recent reaction	
Interferent drugs	Systemic corticosteroid, immunosuppressant drugs (cyclosporin A)	Systemic corticosteroid, immunosuppressant drugs, chemotherapeutic drugs	
Well-prepared sample	Fresh heparinized blood; not more than 24 h	Fresh heparinized blood; not more than 24 h	

Conclusion

Goal for definite diagnosis of severe DHRs contains DHR recognition and culprit drug identification. Severe DHR recognition could be obtained from history and clinical manifestations. However, identification of culprit drug is a tough investigation. Assessment algorithm provides rough result as well as *in vivo* test make a patient with not more or less risk as patient has to be re-exposed with suspicious drug. Therefore, *in vitro* tests could be promising assay to identify causative drug in severe DHRs. Selection of these *in vitro* tests corresponding the mechanisms involved in DHRs is important. Additionally, usefulness of these *in vitro* methods depends performing the test in well-characterized patients with DHR. At present, BAT and LTT are most useful diagnostic assay and influence on the management of SCARs (culprit drug identification and seeking for safety of alternative drug) in clinical practice.

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Conflicts of interest

The authors declare no conflict of interest.

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