Symposium 2: Diagnosis of drug hypersensitivity

# In Vitro Tests for Drug Hypersensitivity reactions

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Immune-mediated hypersensitivity reactions to drugs can occasionally cause a variety of diseases involving the skin, liver, kidney and lungs (1). Drug hypersensitivity reactions are a significant concern for public health, as well as for drug development in the pharmaceutical industry as it is unpredictable, unrelated to the pharmacology of the drug, and potentially severe. It can cause fatal adverse reaction and lead to drug withdrawal from the market and black box label warnings. The frequency, severity, and clinical manifestations of drug hypersensitivity can vary according to the drug, underlying disease, and ethnicity (2, 3).

The Gell and Coomb's classified hypersensitivity reactions into four types (4); drug-induced IgE-mediated type I, IgG-mediated type II, immune-complex mediated type III, and T-cell mediated type IV reactions (Fig. 1). The clinical manifestations and the pathophysiology of drug hypersensitivity are diverse and complex. Types I and IV of drug hypersensitivity are the most frequent and their *in vitro* evaluation system are

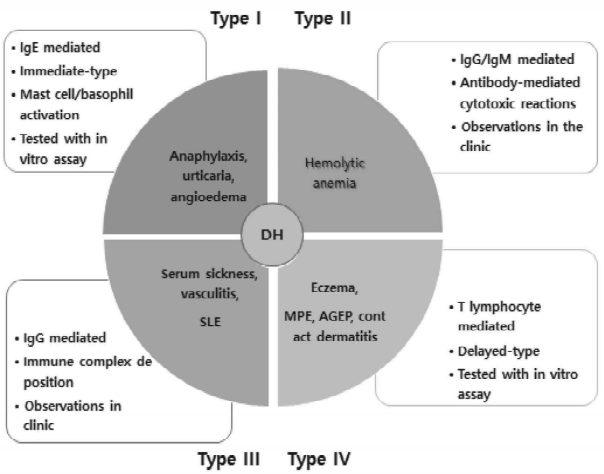


**Fig. 1.** Classification of drug hypersensitivity reactions.

valuable as a complementary test of *in vivo* tests like skin prick test, patch test, intradermal test, and drug provocation test in clinics. An *in vitro* testing for a diagnosis or prediction of drug hypersensitivity reactions seems to be a challenging task.

In this talk, I will briefly summarize the cellular basis of drug hypersensitivity reaction, review the *in vitro* assays that are currently used for characterization of drug hypersensitivity reaction, and discuss some issues related.

## *In vitro* diagnostic tests for drug hypersensitivity reactions

Drug provocation test has been considered the 'gold standard' for diagnosing drug hypersensitivity (5, 6); however, it is often ethically unacceptable as it can trigger severe, life-threatening reactions.

Currently, the diagnosis of drug hypersensitivity is based on a detailed physical examination and medical history. Developing an *in vitro* evaluation system for drug hypersensitivity would safely provide insight into the immunological mechanisms involved in drug hypersensitivity and would enable the simultaneous assessment of immune response to multiple drugs. The main purpose of *in vitro* testing for drug hypersensitivity is to confirm that symptoms result from drug hypersensitivity reaction and to identify the causative drug. Although it is still a challenge, recent progress using *in vitro* approaches to understand the mechanism of drug hypersensitivity opens new possibilities.

*In vitro* testing method depends on whether the initial reaction was an immediate IgE-mediated reaction or a delayed T-cell- mediated reaction (Fig. 2). The cells involved and mediators released during hypersensitivity reactions to drugs can be also assessed as *in vitro* test (7-11).

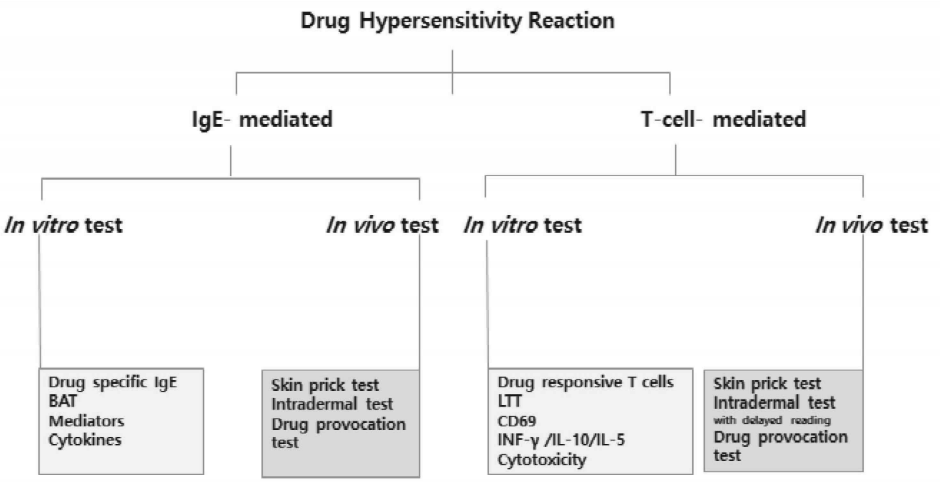


**Fig. 2.** Tests for drug hypersensitivity reactions.

### 1. In vitro testing for immediate type of hypersensitivity reactions

*In vitro* testing for immediate type of drug hypersensitivity involves detection of drug-specific IgE antibodies using radio- or enzyme-labeled anti-IgE antibodies, assessment of basophil activation markers using flow cytometry, and measurement of preformed mediators (12, 13).

1) Quantification of drug-specific IgE
2) Basophil activation test (BAT)
3) Mediator release assays

A few assays for drug-specific IgE measurement are available and most of them have not been thoroughly clinically validated. Basophils and mast cells can be triggered in IgE-dependent or IgE-independent ways, which is responsible for manifestations of the immediate hypersensitivity reaction. BAT relies on a flow cytometric analysis of activation and degranulation markers on the cell surface. The clinical utility of BAT has been suggested for identification of the culprit drug and evaluation of cross-reactivcity. Immediately released mediators such as histamine and tryptase from basophils and mast cells can be assessed.

### 2. In vitro testing for delayed type of hypersensitivity reactions

There are supporting evidence demonstrating essential role of drug-specific T cells in delayed type of drug hypersensitivity such as Stevens-Johnson syndrome, toxic epidermal necrosis and drug-induced hypersensitivity syndrome/ drug rash and eosinophilia with systemic symptoms (14, 15). T cells specifically recognize drugs through their T-cell receptors in an MHC-dependent way; hypersensitivity reaction caused by drugs correlate with the presence of different HLA allele (16, 17). Drugs can stimulate T-cells like haptens via binding to self-peptides (hapten theory) or directly interact with certain T-cell receptors (pharmacologic interaction of drugs with immune receptors, p-i concept) (18).

Delayed-type drug hypersensitivity reactions involve activation of drug-responsive T cells, thus *in vitro* tests are based on effector T-cell responses to the drug (19-23).

1) Lymphocyte transformation tests (LTT; lymphocyte proliferation assay)
2) Cell activation marker and cytokine measurements
3) Drug-induced cytotoxicity assays
4) HLA allele determination

In clinical settings, the patch test and LTT are often used for the diagnostic assessment of drug-specific T-cell responses. The LTT has many advantages. This test is safe and possibly examines multiple drugs at the same time, therefore it might be helpful for the identification of the culprit drug in a patient on multiple drugs. Positive LTT reactions remained to be detected even 1 year after the onset of the drug reaction.

**Table. Advantages and disadvantages of in vitro test available for drug hypersensitivity**

| | Immdiate type | | Delayte type | |
|---|---|---|---|---|
| | Drug-specific IgE | BAT | LTT | ELISpot |
| **Advantages** | use of frozen serum sample | safety | safety | safety |
| | check for cross-reactivity | use of whole blood sample | High specificity | 2 day culture |
| | commercial ImmunoCAP assay | check for cross-reactivity | applicable to multiple drugs | more sensitive than LTT |
| | | commercial BASOTEST, Flow2 CAST | simple readout | applicable in the acute setting |
| | | IgE/non-IgE mediated basophil activation | | |
| **Disadvantages** | not standardized | not standardized | not standardized | |
| | low sensitivity | low sensitivity | low sensitivity | |
| | not available for drug metabolites | technically demanding | fresh blood | high numbers of cells |
| | | | time-consuming procedure (> 6 da | sterile cell culture system |
| | | | technically demanding | |
| | | | correct timing | |
| | | | use of radio-isotope ($^{3}$H-thymidine) | |
| | | | sterile cell culture system | |

## Conclusion remarks

The specificity of currently available *in vitro* testing seems to be very good, but the sensitivity needs to be improved. Sensitivity could be affected by both culprit drug and other intrinsic technical issues. Consequently, a positive result indicates immune mediation of the reaction, while a negative result dose not exclude the possibility of drug hypersensitivity. The diagnostic value of *in vitro* testing can be improved by detecting multiple processes, such as up-regulation of intracellular activation markers, cytokine secretion, and enhanced cytotoxic potential.

Although many *in vitro* tests are available, most *of them* are not standardized and are still primarily research tools. Despite of the limitations for routine use in the clinics, *in vitro* approaches to drug hypersensitivity can give a deeper insight into the immunological mechanisms underlying different types of drug hypersensitivity reaction, which could be informative in future clinical practice.

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