GUIDELINES

In Vitro Diagnosis of Immediate Allergic Reactions to Drugs: An Update

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Abstract

Evaluation of allergic reactions to drugs is difficult because of the poor sensitivity of in vivo tests, which makes controlled administration of the drug necessary to confirm the diagnosis. In vitro tests are important in order to avoid the risks of in vivo testing. In the present review, we describe the different methods for detecting immunoglobulin (Ig) E antibodies that are specific to drugs involved in the development of type I (immediate) reactions. The 2 main in vitro methods are immunoassays and the basophil activation test, both of which have sufficient sensitivity and specificity for the detection of specific IgE antibodies, although with a limited number of drugs, and they have proven complementary to in vivo methods. We show the importance of the allergological workup of the patient within less than 1 year from the occurrence of the allergic reaction in order to obtain positive results in both in vivo and in vitro tests.

Key words: Allergy. IgE. In vivo test. In vitro test. Drug. Immunoassay. Basophil activation test.

Resumen

Actualmente, la evaluación de las reacciones alérgicas frente a medicamentos es un tema complicado debido a que los tests in vivo no presentan una sensibilidad óptima, precisando la administración controlada del medicamento para confirmar el diagnóstico. Con el fin de evitar los riesgos de los tests in vivo, es importante utilizar tests *in vitro*.

En esta revisión, hemos descrito diferentes métodos para detectar anticuerpos IgE específicos frente a medicamentos que están involucrados en el desarrollo de reacciones inmediatas o de tipo I.

Existen hoy en día dos métodos *in vitro* fundamentales, el inmunoensayo y el test de activación de basófilos, que presentan suficiente sensibilidad y especificidad para la determinación de anticuerpos IgE, aunque para un número limitado de medicamentos. Estas pruebas han demostrado ser complementarias a los métodos *in vivo*. Se muestra la importancia de realizar la evaluación del paciente en un periodo de menos de un año desde la reacción alérgica con el fin de obtener resultados positivos tanto en los tests in vivo como *in vitro*.

Palabras clave: Alergia. IgE. Prueba cutánea. Prueba in vitro. Fármaco. Inmunoensayo. Test de activación de basófilos.

Introduction

Allergic reactions to drugs have an immunological basis and may be grouped, according to the classification of Gell and Coombs [1], into 4 types: hypersensitivity reactions (type I), which are mediated by specific immunoglobulin (Ig) E antibodies; cytotoxic reactions (type II); reactions mediated by immune complexes (type III); and T-cell–mediated hypersensitivity reactions (type IV). Of these, the reactions most frequently induced by drugs are type I and type IV.

This classification is consistent with that made in the 1960s by Levine [2], and is based on the timing of the appearance of clinical symptoms after drug intake. Thus, reactions occurring within 1 hour or less are termed immediate reactions, those occurring between 1 and 6 hours after intake are termed accelerated reactions, and those occurring 24 hours or even several days after intake are termed delayed reactions. For diagnostic purposes, accelerated and delayed reactions are currently classified together as nonimmediate reactions [3,4], and both are T-cell–mediated.

Diagnosis of hypersensitivity reactions to drugs is based on a complete and detailed clinical history, including timing, possible causes, and the type of reaction as reported by the patient if the examination is not carried out during the acute phase of the process. This information should be sufficient to establish a diagnosis, although data are often incomplete, and sometimes incoherent or inconclusive; therefore, diagnostic tests should be performed to establish a definitive diagnosis. In the case of immediate reactions to drugs, the methods used are based on in vivo determination of IgE-mediated reactions (skin tests) and on in vitro determination of specific IgE. With in vivo methods, sensitivity is not 100%, even in patients with a clearly positive history, and controlled administration of the drug is necessary to confirm the diagnosis [5]. Given that in vivo testing is not free of the risk of a new allergic reaction, especially when a drug is administered, in vitro diagnostic tests are clearly necessary.

In the present review, we describe the different methods for detecting IgE antibodies to drugs involved in the development of type I (immediate) reactions.

In Vitro Methods

With the exception of determination of specific IgE against a limited number of drugs such as penicillins and muscle relaxants, in vitro methods are not routinely applied in the diagnosis of allergic reactions. However, we believe them to be of interest, as they complement in vivo tests. Technological advances have increased the sensitivity and cost-effectiveness of in vitro determination of drug-specific IgE antibodies, which now offers a series of advantages over skin tests: it does not expose the patient to risk; the results are not affected by concomitant drug treatment, dermographism, or extensive dermatitis; and, in the case of serologic tests, samples may be stored over long periods for future investigation or confirmation.

However, in vitro tests are not without disadvantages. They are generally less sensitive, even though some patients have negative skin test and positive in vitro test results. Furthermore, results are not immediately available, and tests have only been fully developed for a small number of drugs.

Of the in vitro methods currently available, immunoassays for the detection of drug-specific IgE antibodies are the most widely used. In the last few years, there has been increasing interest in cellular methods based on activation of basophils after in vitro stimulation with the culprit drug and quantification of the mediators released (histamine or leukotrienes) in the supernatant or expression of activation markers on the cell surface. Different studies have shown different degrees of agreement between in vivo and in vitro tests.

Immunoassays: Radioallergosorbent Test, Enzyme-Linked Immunosorbent Assay, and Fluorescent Enzyme Immunoassay

Immunoassays are based on detection of antigen (hapten-carrier conjugate) and IgE antibody binding. The most widely used are radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and fluorescent enzyme immunoassay (FEIA). Each has advantages and disadvantages.

The principal condition for these assays is that the hapten molecule or drug must be bound to a carrier molecule. The main limitation is that only a few drugs (ß-lactams, muscle relaxants, and some nonsteroidal anti-inflammatory drugs [NSAIDs]) are able to form adducts and be used in the test. As penicillins have a high capacity to bind to proteins and form hapten-protein conjugates, they have been chosen as a model for in vitro serological testing for drug allergy, and several studies have been published [6-9].

The most widely used immunoassay for the determination of specific IgE is the radioallergosorbent test (RAST) [6], which is based on the binding of a hapten-protein conjugate to a solid phase (cellulose or sepharose) that is then incubated with the serum containing the IgE antibodies specific to the drug. Binding is revealed by incubation with a specific secondary antibody that recognizes the ε chain of the Ig and is labelled with a radioisotope. This technique produces semiquantitative results and has made it possible to analyze critical aspects of in vitro assays such as the importance of carrier molecules, the metabolites involved in the induction of antibodies, specificity, and cross-reactivity.

Several studies have examined the carrier molecules (human serum albumin [HSA], poly-L-lysine [PLL], and a polyalcohol as a spacer) used for the formation of hapten-protein conjugates and their influence on the ability to detect ß-lactam–specific IgE antibodies [7,8]. Results have shown that using PLL and the molecular spacer produces better levels of sensitivity and specificity. These differences arise because of the density of the hapten molecules, which is lower in hapten-HSA conjugates, and because haptens are not always exposed to the antibody for binding.

Studies in patients with allergic reactions to penicillins indicate that RAST has a sensitivity of between 48% and 50%,

and a specificity of 95% [8]. Furthermore, 14% of patients with a confirmed allergy to penicillins had a negative skin test result accompanied by a positive RAST result [9]. This is important when considering whether to avoid controlled administration of the drug to establish a diagnosis.

Little information is available for other drugs, with one study showing evidence of specific IgE antibodies for acetylsalicylic acid [10] and another for pyrazolone (propyphenazone) [11]. Both used HSA as a carrier protein, with a sensitivity of 58% in the latter. The authors also detected specific IgE in patients with negative skin test results and showed that sensitivity can be as high as 96% when both in vivo and in vitro techniques are combined [11].

FEIAs, such as the ImmunoCAP system (Phadia, Uppsala, Sweden), have been validated for allergens and for some drugs (eg, ß-lactams, suxamethonium, chlorhexidine, chymopapain, gelatin, insulin, protamine, and tetanus toxoid). The ImmunoCAP system incorporates a new heterologous calibration scheme and the possibility of quantification based on the use of IgE antibody curves. This enables specific IgE to be quantified in a range of 0.01 kU_A/L to 100 kU_A/L, with a cut-off point of 0.35 kU_A/L for positive results and levels above 0.10 kU_A/L indicating sensitization.

In a study on ß-lactam allergy, ImmunoCAP showed a good correlation with RIA and did not require radioactive isotopes. Sensitivity was 54% and specificity was between 95% and 100% [12]. In a study of patients allergic to amoxicillin [13], Blanca et al showed sensitivity to range between 58% and 68% depending on the hapten used (amoxicillin or benzylpenicillin). Sensitivity increased to 74% when the results from both haptens were taken into account. The study also revealed the existence of a group of patients (42%) with negative skin test results and positive in vitro test results. Specificity was high, ranging between 96% and 100%.

Recently, Fontaine et al [14] analyzed the relevance of IgE antibodies in the serum of ß-lactam–allergic patients and revealed sensitivity and specificity values that were lower than those reported by Blanca et al [13], namely, 37.9% compared to 54%. Comparisons with other non–commercially available RAST techniques showed that the specificity of ImmunoCAP ranged from 83.3% to 100% and sensitivity from 12.5% to 25% [14]. RAST specificity ranged from 66.7% to 83.3% and sensitivity from 42.9% to 75% [14]. These values varied according to the clinical manifestations, with sensitivity and specificity being greater in cases of anaphylactic shock.

The differences in sensitivity between the study by Blanca et al in 2001 [13] and that of Fontaine et al in 2007 [14] indicate a change in the specificity of IgE antibodies in allergic patients, due mainly to changes in ß-lactam consumption patterns. Several authors recommend that the panel of antigenic determinants to be included in the in vitro tests for the evaluation of patients with immediate reactions to ß-lactams must be expanded to include other groups such as the cephalosporins.

The value of IgE quantification by ImmunoCAP in the diagnosis of allergy to other drugs, such as muscle relaxants, has also been analyzed [15], yielding a sensitivity of 68% for rocuronium, 60% for suxamethonium, 88% for morphine, and 86% for pholcodine. Specificity was 100% in all cases except for rocuronium, in which it was 93%. The authors also highlighted the importance of using a correct cutoff to increase the sensitivity and specificity of in vitro tests and observed that this level should be different for each drug.

The sensitivity of immunoassays is generally lower than that of skin tests. Nevertheless, and although positive in vitro test results have been obtained in patients with negative skin test results, in vitro tests should be performed, especially in patients with more severe reactions, in order to avoid the use of challenge tests.

Basophil Activation Test

Techniques based on cellular reactions are gradually being introduced to evaluate immediate allergy to drugs. The most common is the basophil activation test (BAT), which is performed using flow cytometry. This technique is based on the fact that basophils sensitized with IgE at their surface become activated in the presence of the drug and express a high density of markers. The basophils are identified by anti-IgE antibodies and activated by markers such as CD63 or CD203c [16].

There is evidence that the BAT can contribute to the diagnosis of immediate allergy to several drugs, especially ß-lactams, muscle relaxants, and NSAIDs [17-22].

In the case of reactions to ß-lactams, several studies [17-19] have determined the sensitivity of the technique to be around 50%, with a specificity of 93.3%. It is worth noting that in patients with negative skin test results, the sensitivity of BAT can be as high as 60% when the immunoassay is also positive and 14% when it is negative and the drug challenge test is positive.

As for muscle relaxants, one study analyzed 14 patients with anaphylaxis to rocuronium and showed the sensitivity and specificity of BAT to be 91.7% and 100%, respectively. This study also showed that BAT can reveal potential cross-reactions in the evaluation of alternative drugs [20].

In patients with selective allergy to NSAIDs or IgE-mediated reactions, favorable results have been obtained in the analysis of allergic reactions to pyrazolones, which induce IgE-mediated immediate reactions. Two studies [21,22] show that the sensitivity of BAT ranged between 42.3% and 56.7%, with specificity ranging from 83% to 100%. It is important to distinguish between IgE-mediated reactions and cross-reactions to NSAIDs. These reactions occur in patients who react to NSAIDs whose chemical structures are not related; therefore, the mechanism does not involve specific recognition [23]. In these cases, further studies need to be performed to analyze the diagnostic value of BAT.

BAT could therefore prove useful in the diagnosis of type I reactions to drugs. The technique is particularly interesting, due to the difficulty for most drugs to conjugate with a carrier protein, which is the indispensable and limiting condition of immunoassays. Nevertheless, widespread use of this method in clinical laboratories is prevented by the following technical limitations [24-26]:

- The importance of correct sample collection and storage to ensure optimal viability and functionality of the basophils.

- The type of sample (whole blood or separate cells). Although whole blood is more practical and easier to handle, and the presence of all the blood components may better reflect in vivo physiologic conditions, other factors could interfere with the assay. Cell separation, which would avoid these interferences, implies a loss of basophils and the possibility that handling may lead to nonspecific activation.
- The most widely used basophil identification markers include anti-IgE antibodies, even though they are not specific to basophils. CD203c is specific to basophils, yet difficult to use for this purpose, as expression of nonactivated basophils is weak. The use of other markers such as CRTH2, CCR3, and anti-CD123 combined with anti-HLA-DR has been proposed, although this approach has yet to be validated.
- The most frequently used activation markers to date have been CD63 and CD203c. Optimal activation of each depends on the drug being analyzed. Four additional basophil activation markers—CD13, CD107a, CD107b, and CD164—have recently been identified. p38 MAPK has also been added to the list, although further studies are needed to verify its diagnostic validity.

Methodological improvements have made BAT a sensitive and specific technique for in vitro diagnosis of immediate-type allergy and a suitable complement to the in vitro and in vivo tests used to quantify IgE. Since BAT is a cellular method, it resembles more the in vivo pathways that lead to the symptoms, thus making it useful for determining the presence of clinically relevant IgE antibodies when studying cross-reactivity in the quantitative evaluation of residual allergenicity. The protocols used in multicenter studies should now be harmonized and improved in order to allow BAT to enter the mainstream of diagnostic applications.

Tests of Mediator Release: Histamine, Tryptase, and Leukotrienes

Mast cells and basophils are the main cells activated in immediate-type allergic reactions. The release of mediators by these cells following interaction of the antigen with the specific IgE antibodies bound to their surface signals an immediate allergic response [27]. These mediators can be preformed (eg, histamine, tryptase, carboxypeptides, chymases, and heparins, which are released immediately) or must be synthesized de novo (eg, leukotrienes, prostaglandins, and cytokines). Several mediators are indicators of allergic reactions and provide information on the type of response. The most frequently analyzed to evaluate immediate reactions are presented below.

Histamine. Histamine is released by mast cells and basophils a few minutes after the reaction levels are maximal in peripheral blood, although it is rapidly metabolized to N-methyl-histamine, which is eliminated in urine. Therefore, measurement of histamine in peripheral blood is difficult. N-methyl-histamine measured in urine offers a longer time window. These mediators are usually measured using RIA, although other automatic systems exist, such as that developed by Siraganian [28]. One problem is that the levels of N-methyl-histamine in the urine of healthy individuals is variable, with the result that it is necessary to take several serial measurements over at least 24 hours. It must also be borne in mind that levels in healthy individuals may be influenced by diet and circadian rhythm, with physiologic peaks occurring during the early hours of the day. Furthermore, results can vary depending on the drug. Such is the case of quinolones, which have a similar chemical structure to histamine and may produce false-positive results. The difficulty in interpreting results means that this mediator is not considered a useful marker.

Another histamine-based method is the histamine release test, an in vitro approach that analyzes the release of histamine by peripheral blood basophils following the interaction of haptens with IgE antibodies bound to cellular membrane receptors. The histamine released in the supernatant is then measured using RIA. Different authors conclude that, although this technique is useful to discriminate between individuals who are sensitized or not by IgE, it is still not an effective diagnostic test [29,30].

Tryptase. Tryptase is exclusive to mast cells and is therefore useful as an activation marker in immediate allergic reactions [31]. It is measured using the ImmunoCAP system and, although its sensitivity is moderate, its specificity is very high. Healthy individuals have undetectable levels of tryptase in serum and plasma, and in patients with anaphylaxis these rise to over 11.4 µg/L. Levels of tryptase in peripheral blood begin to rise 1 hour after the reaction, and the time they remain increased is variable, ranging from 6 hours to a maximum of 24 hours, approximately, depending on the severity of the reaction. Therefore, tryptase is considered to be a very useful marker for evaluating hypersensitivity reactions to drugs mediated by specific IgE antibodies. Furthermore, it enables the effector cells involved in the process—mast cells—to be identified.

Leukotrienes (LTC_4). Leukotrienes are produced by both mast cells and basophils. CAST-ELISA is one of the methods used to measure the release of LTC_4 from basophils activated with allergens [17]. Data from different studies show that the diagnostic sensitivity of CAST, as compared to the combination of clinical history and skin tests, ranges from 18% for aspirin to 85% for food allergens [32]. Therefore, CAST is not sufficiently sensitive in the diagnosis of IgE-mediated reactions to drugs such as ß-lactams or NSAIDs. It appears to be more useful in the diagnosis of reactions to other allergens, although further clinical studies are needed to confirm this.

While the study of mediators can help to define the immunologic process taking place during immediate allergic reactions and the cells involved (mast cells, basophils, or both), only in the case of the histamine or leukotriene release tests is it possible to obtain additional information on the specificity of allergic patients.

Cross-reactivity

Cross-reactivity can affect the results of in vivo and in vitro tests. It occurs when a drug that has not been previously administered to a patient produces a hypersensitivity reaction due to sensitization to a structurally related component recognized by IgE antibodies or B and T cells [33].

Several studies have analyzed the capacity of different drugs for inducing cross-reactions [34-43]. These include ß-lactams, sulfamethoxazole, and NSAIDs, although ß-lactams have received most attention.

Studies of specificity and cross-reactivity have been carried out using immunoassays (RAST and ImmunoCAP) and, in the case of ß-lactams, RAST inhibition studies [34]. RAST inhibition involves a competitive reaction for the binding of specific IgE antibodies between antigenic structures bound to the solid phase and other structures in the fluid phase. In the case of ß-lactams, cross reactivity has been suggested to be due both to the nuclear structure formed by the ring of the ß-lactam and to the presence of 1 identical side chain [34,35]. The latter phenomenon led to high cross-reactivity between penicillin and cephalosporins in the past, although the use of different and more complex side chains in third-generation and fourth-generation cephalosporins means that such cross reactivity is decreasing [36,37].

Since specific reactions to the side chain were first described, there have been cases of selective responses to amoxicillin that show increased cross-reactivity to cephalosporins sharing the same side chain. Thus, in one study [38], 38% of patients with a selective reaction to amoxicillin experienced a cross-reaction to cefadroxil, which has the same side chain in position R1. Other studies evaluating cross-reactivity between penicillins and cephalosporins by means of skin tests have yielded figures of 12% [39,40]. These results show that penicillin-allergic patients must avoid cephalosporins with the same side chain.

Although it is difficult to determine the role of skin tests in the evaluation of cross-reactivity, a good correlation has been observed with clinical response [40], despite reports of patients with negative skin test results who have shown a positive response after controlled administration of the drug [38].

Cephalosporin-allergic patients can experience 3 types of response: response to cephalosporins with cross-reactivity to penicillin determinants, response to cephalosporins with a negative result for penicillins, and a selective response to the cephalosporin responsible for the reaction [36,41,42]. The cross-reactivity may be caused by induction of antibodies against the common structure of the cephalosporins [36,41-43], although it has also been caused by the presence of the same chemical structure in the side chain in position R1 [41,43]. As for the side chain in position R2, different studies have shown that its role in triggering an allergic response is less important than previously thought, probably because this chain may disappear in the process of cephalosporin fragmentation [36,37,44].

Studies using ImmunoCAP to analyze allergic reactions to rocuronium show that, although this is a good technique for detecting specific IgE antibodies, inhibition studies do not predict clinically relevant cross-reactivity [15]. The same observation has also been made in patients with allergic reactions to ß-lactams and on the basis of analyses performed with BAT [17,18].

Importance of the Time Interval Elapsed in the Detection of IgE

When carrying out an allergological workup in patients with immediate reactions to drugs, it is important to bear in mind that the rate of negativization of IgE increases with time from onset, thus reducing the sensitivity of the methods used. This is because specific IgE antibody levels tend to decrease over time in the absence of new contact with the hapten that induced the reaction. This factor is important when analyzing reactions to drugs, although less so in allergens, especially aeroallergens, as it is very difficult to avoid continuous contact with the antigenic determinant responsible for the reaction.

The reduction in specific IgE antibody levels over time has been shown with both in vivo and in vitro methods and in reactions to various types of drugs, such as penicillins, muscle relaxants and, more recently, pyrazolones [22,45,46]. One study comparing the negativization rates of 2 in vitro techniques (RAST and BAT) in amoxicillin-allergic patients found that, 1 year after the reaction, only 12% of patients continued to be BAT-positive, while 22% remained RAST-positive [45]. The authors observed a high negativization rate in the in vitro tests used to measure specific IgE, especially with BAT. Furthermore, this same study used a skin test to analyze the importance of contact with the hapten in the reduction of antibody levels and found that, while this influences the reduction in negativization rates for RAST, it appears to have no influence on BAT.

The negativization rate in BAT has also been studied in other drugs, such as muscle relaxants, where it has been observed that sensitivity was 85% when patients were evaluated within a period of 3 years following the reaction, but fell dramatically to 47% when patients were evaluated more than 4 years after the reaction [46].

In a recent study assessing the role of BAT in the diagnosis of IgE-mediated immediate reactions to pyrazolones, patients were followed for 30 months, and it was found that negativization of BAT results occurred in 60% [22].

The main factor influencing the negativization of these in vitro tests appears to be the reduction in IgE antibody levels. It has also been reported that the speed of this reduction depends on the specificity of the antibodies, in such a way that the more specific they are, the faster their levels fall over time, although further studies are required to confirm this [45].

Therefore, an allergological workup should be performed within less than 1 year from the occurrence of the allergic reaction, as this time interval is critical for obtaining positive results.

Conclusions

At present, the 2 in vitro methods available are immunoassays and the BAT, and both have sufficient sensitivity and specificity for the detection of specific IgE antibodies to drugs. However, immunoassays are limited in that they can be applied to only a few types of drugs, especially those that are capable of binding to proteins. Although the sensitivity of in vitro tests for drugs is not very high, evidence exists for both immunoassays and BAT in cases with a history of immediate reactions to ß-lactams, and negative skin test but positive IgE results [9,17,18,47]. Such evidence shows that the results obtained from in vivo and in vitro studies are not fully comparable. This is probably because in each of the methods the antigenic determinants included

for the detection of specific IgE antibodies are not always the same [48]. Therefore, combined use of both in vitro and in vivo studies will improve the sensitivity of allergological workups and will remove the need for challenge tests.

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