Provocation tests in drug allergy

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ABSTRACT

Almost two-thirds of patients who visit a doctor for a reaction that is diagnosed as a 'drug allergy' (DA) are not allergic. This false claims of drug allergy leads to unnecessary avoidance of first choice drugs, exposes the patient to less effective and potentially more toxic drugs, increases healthcare costs, and contributes to the development of antibiotic resistance. Therefore, a drug allergy evaluation at a specialized center must be offered to all patients with suspected drug allergy. Drug allergy evaluation comprises patient's history, *in vivo* skin testing (prick, intradermal and patch tests), *in vitro* and provocation tests. Skin tests have been validated for penicillins, muscle relaxants and carboplatin and can also be useful with some other drugs, including iodinated contrast media (ICM) and anticoagulant drugs. Most *in vitro* studies have not been sufficiently evaluated to be used as standard investigation outside the context of prospective studies. Drug provocation tests (DPT) are often considered an unnecessary and dangerous method for drug allergy evaluation. In this reviews we present the updated indications for DPT and the safety data concerning DPT. We conclude that DPT is a safe procedure if performed in specialized centers. There are no accurate alternatives in most cases. DPT remains the gold-standard to validate alternative diagnostic methods.

INTRODUCTION

An adverse drug reaction (ADR) is defined by the World Health Organization as a noxious and un-intended response to a drug that occurs at a dose normally used in man[1]. ADRs can be classified into reactions which may affect anyone (type A) and reactions which affect only susceptible individuals (type B). According to the Nomenclature Review Committee of the World Allergy Organization, drug allergy (DA) refers to a hypersensitivity reaction for which a definite immunological mechanism, either IgE or T-cell-mediated, is demonstrated[2]. ADRs that clinically resemble an allergy but for which an immunological process is not proven should be classified as non-immune hypersensitivity reactions[2].

It is important, however, to distinguish between immediate and nonimmediate reactions[3]. The former occur within the first hour after the last drug administration and are manifested clinically by urticaria, angioedema, rhino--conjunctivitis, bronchospasm and anaphylactic shock. Nonimmediate reactions occur more than 1h after last drug administration. The main nonimmediate reactions are maculopapular eruptions and delayed-appearing urticaria/angioedema. In addition, drugs can elicit fixed eruptions, exfoliative dermatitis, acute generalized exanthematous pustulosis (AGEP), drug reaction with eosinophilia and systemic symptoms (DRESS), Stevens-Johnson syndrome, and toxic epidermal necrolysis (TEN)[4]. Immediate allergic reactions are usually IgE-mediated, and have been extensively studied, whereas the mechanisms involved in nonimmediate reactions seem to be more heterogeneous[4].

EPIDEMIOLOGY OF DRUG HYPERSENSITIVITY

Drug hypersensitivity reactions (DHRs) represent about one third of ADRs which can affect 7% of the general population and up to 20% of hospitalized patients besides being responsible for as many as 8% of hospital admissions, followed by nonsteroidal anti-inflammatory drugs (NSAIDs). Antibiotics are the most frequent culprit drugs. In most cases reactions are not declared but reported numbers can also be inflated by the lack of a definite diagnosis[5].

A cross-sectional survey of a general adult population from Porto[6] found a 7.8% (181 of 2309) prevalence of self--reported DA, of which 4.5% were to penicillins or other betalactams, 1.9% to aspirin or other NSAIDs and 1.5% to other drugs. Similar results were found among university students using a comparable methodology[7]. A cross-sectional survey assessing the life occurrence of ADRs and self-reported DHRs in 1426 parents of children attending the outpatient clinic of Hospital Maria Pia found a prevalence of 10.2% for ADRs and 6% for DHRs[8]. Most of the suspected DHRs were non-immediate cutaneous events attributable to betalactam antibiotics and occurred in very young children.

IMMUNOPATHOLOGY OF DRUG HYPERSENSITIVITY REACTIONS

Although drugs are capable of inducing all the types of immunological reactions initially described by Gell and Coombs, they are in the vast majority immunoglobulin-E- or T-cell mediated reactions. Very often the allergic determinants are unknown, and sometimes several allergic mechanisms and nonallergic mechanisms may be responsible[9]. Three major but not mutually exclusive models are proposed to explain drug-induced immune reactions: the hapten (and pro-hapten)-carrier concept, the danger signal concept and the p-i (pharmaco-interaction with immune receptors) concept[10].

The hapten-carrier concept postulates that small compounds become antigenic for both B- and T-cells after covalent binding to larger proteins. Heterologous sera, some enzymes (chymopapaïne…) and hormones (insulin…) have sufficient molecular weight to be immunogenic in a native state. Most other drugs have a low molecular weight and are immunogenic only because they haptenize endogenous proteins. Some drugs are intrinsically reactive (hapten concept), while other drugs require enzymatic or non-enzymatic conversion to a reactive intermediate (pro-hapten concept)[10].

The danger signal concept, initially proposed by Matzinger, states that an immune response can only be mounted when antigen-presenting cells (APC) receive activating signals from stressed or damaged cells[11]. Thus, drug-related cytotoxicity may also be of importance. According to this concept, the immune response to a drug-derived antigen requires the presence of co-stimulatory signals and cytokines, which propagate and determine the type of immune response. The "danger signal" might result from chemical, physical or viral stress[12]. Recent findings indeed suggest that the drug itself can give the danger signal as well.

However, many drugs are not reactive and can still be immunogenic. Thus, Pichler has described a model of T-cell stimulation by drugs, termed pharmacological interaction with immune receptors (p-i concept)[4,13]. He states that certain drugs would interact with T-cell receptors and MHC molecules in a direct way, without covalent binding to the receptor and without processing by APC. The interaction would be sufficient to stimulate T-cells if an MHC interaction was provided. Some proofs to this concept have been published, but is still a matter of debate.

Finally, other drugs are not taken by the immune system and induce nonimmunological DHRs. Pharmacogenetic variations may well explain this and future studies will address that issue too.

CONTINUOUS NEED FOR DRUG ALLERGY EVALUATION

Almost two-thirds of patients who visit a doctor for a reaction that is diagnosed as a `drug allergy' are not allergic[14]. Pharmacovigilance algorithms are not accurate for the diagnosis of DHRs and cannot replace drug allergy testing.[15]

In the largest published series[16], 898 patients with suspected drug allergy referred to a specialized center were tested with the suspected drugs and a total of 1372 DPTs were performed. Only 241 (17,6%) were positive, allowing to exclude drug allergy in the majority of patients.

Also in our study of outpatients from Hospital Maria Pia, when skin prick tests (SPT), intradermal (ID) tests and/or oral challenge were undertaken in children who gave a plausible history of drug allergy, 94% were able to tolerate the drug[8]. Therefore, many children can have an unnecessary lifelong label of drug allergy possibly leading to the prescription of less effective and more costly treatments.

We have recently reviewed all published reports of suspected allergy to macrolides[17]. Overall only 94/199 reports were supported by any drug allergy evaluation (SPT in 38, ID in 12, Patch tests in 52, DPT in 56 and other tests in 16). This review also reflects the lack of drug allergy evaluation even in published reports.

As unnecessary avoidance of first choice drugs exposes the patient to less effective and potentially more toxic drugs, increases healthcare costs, and contributes to the development of antibiotic resistance[18], a drug allergy evaluation at a specialized center must be offered to all patients with suspected drug allergy.

DIAGNOSIS OF DRUG HYPERSENSITIVITY REACTIONS

The diagnosis of DHRs is difficult and drug-allergic reactions are surely underdiagnosed. This difficulty is related to the following factors: *1)* the variability of the clinical picture; *2)* insufficient understanding of the pathophysiologic reactions of most DHRs; *3)* some weakness of the classification of allergic reactions into four types according to Gell and Coombs, which is helpful for some drug-allergic reactions but fails to explain some more common and/or more severe forms of drug allergy; *4)* the limitation and/or lack of standardization of the available *in vivo* and *in vitro* test procedures to detect DHRs. In view of these difficulties, most doctors restrict themselves to a careful clinical history, reference books, local agencies monitoring ADRs, and/or databanks describing side effects attributed to a certain drug. However, only a formal diagnosis of drug allergy allows one to bring into play the measures required for prevention and treatment[19].

Drug allergy evaluation work-up involve patient's history, *in vivo* skin testing (prick, intradermal and patch tests), *in vitro* laboratory tests and provocation tests[20].

Clinical history

A detailed history is of paramount importance for the question of whether a certain disease reflects DHRs as well as the question of which drug is causing it. To facilitate the recording of an appropriate history and to harmonize this procedure in Europe, members of ENDA (European Network of Drug Allergy, which is the interest group on drug allergy of the European Academy of Allergy and Clinical Immunology) have developed a questionnaire which might provide a guide in this rather difficult area of clinical medicine[19].

Skin tests

Different types of skin tests (prick, intradermal and patch tests) could be applied according to the suspected pathomechanism of the DHRs[21-24]. Skin tests have been validated for penicillin, muscle relaxants and carboplatin[25-28] and can also be useful for some other drugs, including iodinated contrast media (ICM) and anticoagulant drugs[29]. However, for most drugs the relevant immunogen (intermediate metabolite) is unknown and therefore the predictive value of skin testing remains undetermined. Both false--positive and false-negative results may occur[30].

In immediate, possibly IgE mediated, reactions (manifested by anaphylaxis, bronchospasm, conjunctivitis, rhinitis, urticaria/angioedema) SPT and/or ID tests with reading after 20 minutes can be used[20]. For non-immediate reactions, occurring more than one hour after drug intake, manifested mainly by cutaneous symptoms, patch tests and/or late-reading ID tests might be useful[20]. ID tests are more sensitive than SPT, but have a higher rate of false positive results and might even lead to an anaphylactic reaction in IgE-dependent reactions[20].

Drugs interfering with skin tests (mainly anti-histamines, oral and topical corticosteroids) should be discontinued prior to skin testing. Patients being tested after a drug induced anaphylactic reaction should be out of ß-adrenergic blockers as they may interfere with treatment of a possible systemic reaction elicited by the skin tests. Skin tests are not innocuous and some patients might experience systemic reactions after percutaneous and epicutaneous skin testing[20]. Patients that have experienced severe DHRs (requiring hospitalization) or life threatening reactions including anaphylaxis, severe skin reactions (*e.g.* Stevens Johnson Syndrome) or systemic reactions are at a higher risk, even if there is a long time interval between DHR and drug allergy testing[31]. In such high risk patients an individual risk-benefit assessment has to be done. If tests are to be performed, they should be initiated using a higher dilution of the test preparations (e.g. 1/10 – 1/10000)[20].

For skin testing, preparations for parenteral (mostly i.v.) route should be used, using 0.9% NaCl for dilutions. For ID tests, sterile solutions are obligatory. When drugs are only available as tablets, these can be smashed in a mortar and diluted with saline. Test concentrations should be given in mg drug/ml vehicle. For patch tests, substances should be diluted in 0.9 NaCl or in petrolatum, depending on the solubility and toxicity of the preparation[20].

Skin tests should be performed with a concentration that does not cause a reaction in a sufficient number of controls. For some drugs the optimal test concentrations have been published while for others they remain unknown and must be determined. The optimal test concentration is the highest concentration of a particular drug, which does not give any skin reaction in a group of exposed and unexposed controls, but may originate a positive reaction in patients with drug allergy[20].

The negative predictive value of skin tests is generally low. This may be partly due to the fact that physiologic metabolites rather than the active drug itself are responsible for the reaction and because many drugs are haptens, which have to be conjugated with a carrier protein before becoming an allergen. Thus, a negative skin test to a drug alone is unreliable for ruling out drug allergy. In the case of a negative skin test, progression to more hazardous DPTs should be considered[20]. The positive predictive value of a skin test tends to be high, provided that a sufficient number of controls have been tested negative with exactly the same methodology[32].

When penicillin is suspected as the cause of an immediate reaction, skin testing with the major determinant penicilloyl polylysine (PPL) and the minor determinants of penicillin, penilloate, penicilloate, benzyl penicillin (minor determinant mix or MDM) and amoxicillin provide useful information if positive.[33] Standardization of skin test reagents has been attempted for penicillin with PPL and MDM determinants with the re-introduction of a commercial kit. Comparison between the previous and the current commercial preparation on a database group of known penicillin sensitive patients has shown comparable results[34].

Details on skin test procedures in the diagnosis of DHRs have been published by ENDA[20].

Laboratory tests

Testing for **specific IgE** in sera is only available for a limited number of drugs. These tests have unknown sensitivity and specificity as they require validation against sera from proven cases. Serum-specific IgE is therefore useful when it is positive, but negative results are difficult to interpret[35]. A further disadvantage is that potentially cross--reacting drugs or other co-administered drugs and reagents cannot be tested routinely at the same time and therefore skin testing for drug allergy is preferable[30].

Cellular allergen stimulation test (CAST) for the measurement of leukotrienes after peripheral blood leukocyte stimulation, **basophil histamine release tests** and **basophil activation tests** are also available. Although CAST is commercially available it has not been sufficiently evaluated to be recommended as a standard investigation outside the context of prospective studies. Basophil activation markers using fluorescence activated cell sorter analysis are currently being evaluated for certain types of DHRs but currently there seems to be no evidence of any advantage of these tests over skin testing[36].

Flow cytometry detection of specific surface markers with monoclonal antibodies (CD63 and CD203c) can contribute to the diagnosis of anaphylactic reactions from several drugs, particularly muscle relaxants, betalactams, and NSAIDs. For betalactams the sensitivity is over 50% and specificity over 90%[37,38].

The **lymphocyte transformation test (LTT)** measures the proliferation of T cells to a drug *in vitro* – from which one concludes to a previous *in vivo* hypersensitivity reaction. The usefulness of the LTT has been confirmed by the generation of drug-specific T-cell clones and the finding that drugs can directly interact with the T-cell receptor, without previous metabolism or need to bind to proteins[39]. The main advantage of this test is its applicability with many different drugs in different immune reactions, as drug-specific T cell are almost always involved in allergic drug reactions. Its main disadvantages are that an *in vitro* proliferation of T cells to a drug is difficult to transfer to the clinical situation and that the test *per se* is rather cumbersome and technically demanding. In addition, its sensitivity is limited (for betalactam allergy it is in the range of 60-70%)[39], i.e. closed to that of skin tests.

DRUG PROVOCATION TESTS (DPT)

A drug provocation test (DPT) is the controlled administration of a drug in order to diagnose DHRs. DPTs are performed under medical surveillance, whether this drug is an alternative compound, or structurally/pharmacologically related, or the suspected drug itself[40]. DPT is widely considered to be the "gold standard" to establish or exclude the diagnosis of DHR to a certain substance, as it not only reproduces allergic symptoms but also any other adverse clinical manifestation irrespective of the mechanism. It thus has advantages over all other test procedures and even can prove or disprove the clinical relevance of test results obtained with other *in vivo* and *in vitro* test methods. But DPT should be performed only if other, less dangerous test methods do not allow relevant conclusions and if the outcome might thus help to clarify an otherwise obscure pathologic condition[40].

DPT with a suspected drug should not be performed in pregnant women or in patients at increased risk due to co-morbidities, like acute infections or uncontrolled asth-

ma, or underlying cardiac, hepatic, renal, or other diseases, where exposure might provoke a situation which is beyond medical control[40]. In most circumstances it is difficult to justify DPT with drugs that are nowadays mostly obsolete like sulfonamides (except in HIV-positive persons) or substances with debatable value like many herbal products or "lifestyle drugs"[41]. DPT should never be performed in patients who have experienced severe, life-threatening immunocytotoxic reactions, including vasculitic syndromes, exfoliative dermatitis, erythema multiforme major/Stevens--Johnson syndrome, drug induced hypersensitivity reactions (with eosinophilia)/ DRESS and toxic epidermal necrolysis[40].

Ideally, DPT might be performed via different routes of administration, including oral, parenteral (i.v., i.m., s.c.) and topical (nasal[42], bronchial[43,44], conjunctival[45] or cutaneous[46]). In principle, the drug should be administered in the same way as it was given when the reaction occurred, the oral route is favored if possible, since absorption is slower and developing adverse reactions can thus be treated earlier as compared to DPT performed by the parenteral route[40].

Any medication that might interfere with DPT challenge (especially anti-histamines, some antidepressants and systemic glucocorticosteroids) should be avoided before challenges.

Before performing a DPT, an individual assessment of risk/benefit must be done and challenge only performed if this ratio is acceptable[41]: the drug must be important, *i.e.* it has to be substantially more effective than other alternatives, the condition being treated must be serious, no alternative testing method is available or the results are inconclusive. The patient must be informed of the consequences of both the use of alternative treatments and the risks involved in DPT. The patient should give written informed consent for the test[40].

DPT should be regarded as a serious and potentially dangerous test procedure. It is essential to have well--trained medical staff, that is immediately available in case of emergency, and facilities for continuous monitoring of the patient's condition. Intravenous access and intensive care room access/emergency treatment should be available depending on the severity of the previous reaction and the type of drug. Procedures like spirometry, monitoring of blood pressure, pulse and vital signs must be performed according to the patient's individual situation. Evolution of life-threatening reactions may make fast access to intubation essential[40].

DPT should be performed placebo-controlled, single blinded, and, in certain situations where psychological aspects may prevail, even double blinded. This is of utmost importance, since even in healthy students and hospital staff without any medication but placebo capsules, 41% reported (mostly subjective) symptoms like sedation, irritation, but even nasal congestion, fever, exanthema or urticaria within a 3-day observation period[47].

Generally one should start with a low dose, carefully increasing this and stopping as soon as the first objective symptoms occur. If no symptoms appear, the maximum single dose of the specific drug must be achieved, and the administration of the defined daily dose is desirable[40]. Depending on the drug and the patient's response threshold, DPT may be completed within hours, days or, occasionally, weeks[48].

Patients should be observed as long as severe exposure--related reactions may be expected. This depends on the type of previous drug reaction, the drug under investigation and the individual situation of the patient[49]. If mild reactions had occurred, observation after stabilization is recommended for at least 2 hours. After severe reactions, hospitalization is recommended because of the possibility of biphasic episodes that can be lethal if not recognized early and treated adequately[50]. After discharge, the patient should be equipped with an adequate emergency treatment if further symptoms seem possible (antihistamines, betamimetics, glucocorticosteroids).

A DPT can be termed positive if it reproduces the original symptoms[40]. Although considered the gold--standard for the diagnosis of drug allergy, DPT can result in false positive reactions (due to psychological

symptoms, to preexisting symptoms like chronic urticaria, drug-induced exacerbation of preexisting disease or self-infliction). More important, a negative test does not always prove tolerance for future situations, since there might be false negative results (due to intake of antiallergic drugs, missing co-factors like co-medication, viral infections, physical exercise or insufficient dose exposure)[40].

General considerations on DPT in diagnosis of DHRs have been published by ENDA[40].

MYTHS AND FEARS ABOUT DRUG PROVOCATION TESTS:

There are two main reasons why people fear DPT:

- There are safer and accurate alternatives for drug allergy diagnosis;
- DPTs are extremely dangerous and the risk/benefit ratio is unacceptable.

1. There are safer and accurate alternatives for drug allergy diagnosis

As it has been previously mentioned, skin tests, both with immediate and delayed readings, have been validated for a few drugs (betalactams, muscle relaxants, carboplatin)[29].

A recent update on betalactam hypersensitivity by the ENDA group has been published[51] and states that new evidence suggests that skin test sensitivity is lower than previously reported (in studies by Lammintausta et al[52], amoxicillin (AX) gave positive reactions to patch tests in 10/247 (4%) and benzylpenicillin (BP) in 6/152 (3.9%). The authors concluded that due to the decreased skin test sensitivity in non-immediate reactions to betalactams, many patients need a DPT with the culprit drug to be diagnosed. The sensitivity of skin testing may be higher in more severe reactions, such as desquamative exanthemas.

The absolute requirement for oral provocation in patients with positive clinical history and negative skin tests for betalactams has been recently re-emphasized[53]. In this study, 32.9% of allergic patients had negative skin tests but were positive on provocation. In a subsequent study of 1218 patients with suspected betalactam allergy, 21.1% had a true betalactam allergy confirmed by skin tests (178, 69.3%) or by drug provocation (79, 30.7%); 17.4% of the patients with negative skin tests to major and minor penicillin determinants were positive for a betalactam. The authors conclude that in the diagnosis of betalactam allergy, if all skin tests are negative, skin tests with other determinants and provocation tests under strict surveillance are mandatory[54].

Torres et al. performed DPT in 89 patients with history of immediate reaction to penicillins but negative SPT and CAP-IgE-FEIA to classical and side chain penicillin determinants[55]. After DPT, 49/89 (55%) patients developed immediate responses and were considered allergic. The authors conclude that an important number of subjects are not correctly identified if only skin test and/or CAP-FEIA are used.

We can conclude that skin tests are not enough. In specialized centres it might be prudent to confirm the diagnosis by use of a provocation test, in order to gain and publish information about SPT validity with each specific drug and clinical manifestation[20].

2. DPTs are extremely dangerous and the risk/benefit ratio is unacceptable

In 1372 DPTs performed in patients referred to a drug allergy center with previous immediate reaction to a drug, only 241 (17,6%) were positive[16]. DPT reproduced the initial symptoms, but milder and of shorter duration. All reactions promptly responded to standard treatment.

Williams et al have published results of DPT in 210 consecutive patients referred with suspected aspirin-exacerbated respiratory disease[56]. Of 147 patients who reported seeking acute medical care for their historical aspirin/NSAID-induced asthma attacks, 101 (69%) were treated in an emergency department and released, and 46 (31%) required hospitalization. During DPT in these 147 subjects, 23 (16%) had a 20% to 29% decrease and 14 (10%) had a 30% or greater decrease

in FEV1 values from baseline. Of the 46 patients previously hospitalized for aspirin/NSAID-induced asthma attacks, 9 (20%) had a 20% to 29% decrease and 6 (13%) had a 30% or greater decrease in FEV1 during DPT. By contrast, of the 63 patients who treated their prior aspirin/NSAID-induced reactions at home, 5 (8%) had a 20% to 29% decrease and 5 (8%) had a 30% or greater decrease in FEV1 during DPT (no significant difference between groups).

The authors concluded that the severity of the historical aspirin/NSAID induced asthma attack was not predictive of asthma severity during DPT. These data provide further reassurance regarding the safety of outpatient aspirin desensitization even in patients who report previous reactions severe enough to warrant acute medical care, hospitalization, or admission to the ICU.

Moreover, we did not find any report of fatal reactions on DPT with drugs (we used the following query (Drug Challeng* [TIAB] OR drug provocat* [TIAB]) AND ("Drug hypersensitivity" [MH] OR "Drug hypersensitivity" [TIAB] OR Allerg*[TIAB]) AND ("death" [MH] OR "deathl" [TIAB] OR "fatal" [MH] OR "fatal" [TIAB]) in Pubmed).

We agree that DPT should be regarded as serious and potentially dangerous tests that must be performed by well-trained medical staff. But the same is true for skin tests. Skin tests are not risk free. Systemic symptoms induced by skin testing occurred in 32/290 (11%) patients with history of immediate allergy to penicillins[57]). Even anaphylaxis has been reported[58].

CONCLUSION

DPT is a safe procedure if performed under the most rigorous surveillance conditions in specialized centers with on-site intensive care facilities. It remains the gold standard for the identification of an eliciting drug when allergologic tests are negative, not available, or not validated. DPT allows not only to confirm DA diagnosis, but also to exclude it in the majority of patients with a history of hypersensitivity reaction but with negative results in skin or in *in vitro* tests.

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