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Diagnosis of allergic reactions to sulfonamides

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Adverse reactions to drugs are a significant cause of both morbidity and death. Recently, Lazarou et al. (1) estimated the incidence of serious and fatal adverse drug reactions (ADRs) by conducting a meta-analysis. They evaluated 39 studies, done in the USA from 1966 to 1996, that prospectively monitored and recorded all ADR occurrences in hospitalized patients. It was found that the overall incidence of serious ADRs was 6.7% of hospitalized patients and that the overall incidence of fatal ADRs was 0.32%. When both serious and nonserious ADRs were considered together, the percentage of ADRs more than doubled to 15.1% of hospitalized patients.

Drug reactions can be elicited by any therapeutic agent. However, some drugs are more likely to cause reactions than others. While adverse reactions to sulfonamides occur in 4–6% of individuals without HIV infection, they take place in 50–60% of patients with AIDS, causing significant morbidity and deaths in this population (2–11). The reactions seen differ clinically, suggesting that diverse mechanisms are involved. Toxic metabolites, in combination with immunologic and metabolic factors, may play important roles in the elicitation of these reactions, along with genetic predisposition and acquired glutathione deficiency (12–18).

Sulfonamides are metabolized in the liver by one of two pathways. *N*-acetylation yields nontoxic metabolites, while cytochrome P-450-catalyzed *N*-oxidation produces reactive intermediates. These reactive metabolites are reduced by glutathione and then excreted. However, when the capacity for glutathione conjugation is exceeded, reactive metabolites may be directly cytotoxic or may haptenate protein carriers (13, 16, 18–20). The clinical reactions that result are diverse, with sulfamethoxazole (SMX) alone causing over 20 types of reactions (Table 1).

Unfortunately, at this time, we have few tools to help in the diagnosis of sulfonamide reactions. Much of the

problem lies in the fact that, for many of these reactions, the mechanism responsible for their elicitation is not known. Therefore, without knowledge of the underlying mechanism, selection of appropriate diagnostic tests is difficult.

Currently, the diagnosis of drug allergy, including sulfonamide allergy, is based primarily upon a detailed clinical history and physical exam. In addition, despite the fact that limited diagnostic tests are available, some *in vivo* and *in vitro* tests do exist, and these may be useful in demonstrating that sensitization to a drug has occurred. The diagnostic tests that may be useful in the diagnosis of sulfonamide reactions are outlined in Table 2.

Skin testing for immediate hypersensitivity reactions

For those reactions that appear to be IgE mediated (anaphylaxis, urticaria, angioedema), skin testing may be used to detect sulfonamide-specific IgE antibodies. Using a multivalent skin test reagent, SMX-poly-L-tyrosine, we demonstrated the presence of SMX-specific IgE antibodies in SMX-allergic non-HIV-infected individuals (22). As shown in Table 3, 34 patients with histories of immediate hypersensitivity reactions to SMX and six individuals with no history of SMX allergy were prick tested with the SMX conjugate at a concentration of 1 mg/ml. While none of the control individuals were skin test positive, 10 of 34 (29%) of those with histories of immediate hypersensitivity reactions to SMX had positive prick tests. The fact that only 29% of the history-positive patients were skin test positive was not an unexpected finding. Most of the patients tested had a remote history of SMX allergy, and it is known that, like penicillin-specific IgE antibodies, SMX-specific antibodies wane over time (22).

Table 1. Sulfonamide-associated reactions (21)

Anaphylaxis	Angioedema	Aphthous stomatitis
Bullous eruption	Erythema multiforme	Erythema nodosum
Erythroderma	Exanthema	Exfoliative dermatitis
Fixed drug eruption	Flushing	Lichenoid eruption
Lupus erythematosus	Photosensitivity	Pruritus
Psoriasis	Pustular eruption	Stevens-Johnson syndrome
Sweet's syndrome	Urticaria	Toxic epidermal necrolysis
Vasculitis	Hypersensitivity reaction	Serum sickness

Lymphocyte transformation

The lymphocyte transformation test (LTT) may be helpful as part of the diagnostic evaluation of patients who have presumed drug allergies. The demonstration of a proliferative response by peripheral blood lymphocytes from a drug-allergic patient, cultured in the presence of the presumed culprit drug, indicates that drug-specific T-cell sensitization has occurred (23–25). In Nyfeler & Pichler's (25) study of 100 patients who had clear drug allergy histories, the LTT was positive in 78. In addition, 15 of 102 patients who did not have a drug allergy history also demonstrated a positive LTT. Thus, in this study, the sensitivity and specificity of the LTT were 78% and 85%, respectively. Among the group of drug-allergic individuals evaluated, six had SMX-associated drug reactions, and in all six of these individuals, a positive LTT could be demonstrated (100% sensitivity). In light of these results, the LTT may prove to be a useful tool in the diagnosis of sulfonamide allergy.

Sulfonamide-induced lymphocyte cytotoxicity

Sulfonamide metabolites, particularly the nitroso metabolite (26), have been shown to be directly cytotoxic to lymphocytes (12, 13, 19, 27–30) *in vitro*. Thus, in light of this finding, it has been hypothesized that SMX-induced severe reactions, in some individuals, may result from a defect in the detoxification of culprit drug-reactive metabolites. To test this hypothesis, Wolkenstein et al. (31) challenged lymphocytes from 13 SMX-allergic patients with reactive metabolites that had been generated *in vitro*. The SMX-induced cutaneous reactions experienced by the patients examined included toxic epidermal necrolysis, Stevens-Johnson syndrome, fixed drug eruptions, and exanthema. When the *in vitro* incubation mixture included lymphoid cells from the patients, a sulfonamide drug, and a complete

Table 2. Sulfonamide reactions: diagnostic tests

Skin testing with SMX-conjugate or native drug (immediate hypersensitivity reactions only)
Lymphocyte transformation assays
Lymphocyte cytotoxicity assays
Assays for detecting antibodies to endoplasmic reticulum luminal proteins (for delayed-onset hypersensitivity-type reactions)

microsomal oxidation system, it was found that lymphoid cells from 8/13 patients were significantly more susceptible to the toxic effects of the drug metabolites generated than were those from control nonallergic individuals.

The results of this study, as well as the results of others (12, 13, 19, 27–30), support the hypothesis that sulfonamide reactions may indeed result from a defect in drug detoxification. However, while lymphocyte cytotoxicity testing may someday be used in the retrospective evaluation of sulfonamide ADRs, more studies need to be performed to determine the usefulness of this potential diagnostic tool. At this time, the sensitivity appears to be low (62% in Wolkenstein et al.'s study [32]). In addition, since this test is not easy to perform, it remains only a research tool currently.

Sulfonamide-induced microsomal antibody production

Like sulfonamides, halothane and aromatic anticonvulsants also are metabolized by cytochrome P450 isoenzymes to reactive intermediates. Interestingly, in sera from patients with halothane hepatitis and anticonvulsant-induced hypersensitivity syndromes, antibodies that recognize native, but not drug-modified, endoplasmic reticulum proteins have been identified (33, 34). More recently, Cribb et al. (35) investigated whether patients with delayed-onset sulfonamide hypersensitivity reactions had similar antibodies. They evaluated:

1) 21 patients who had experienced a delayed-onset, sulfonamide-induced, hypersensitivity-type syndrome that was characterized by fever, skin rash, and multiorgan toxicity
2) 11 control individuals

Table 3. Wheal and flare skin-test responses to SMX-polytyrosine (22)

Subjects	No. tested	No. positive	% Positive
Controls	6	0	0
Allergic patients	34	10	29

3) 18 patients who had experienced a sulfonamide-associated adverse event that was not consistent with a hypersensitivity reaction.

With rat liver as a source of microsomal protein and an immunoblotting system, 17/21 patients had antibodies that recognized one or more native endoplasmic reticulum proteins. None of the control subjects and only 1/8 patients who had nonhypersensitivity-like reactions had antibodies against microsomal proteins. Only one patient possessed antibodies that recognized the sulfonamide hapten.

The results from this study suggest that patients with delayed-type hypersensitivity reactions to sulfonamides may possess antibodies that recognize specific microsomal protein epitopes. Interestingly, the antibodies that were detected in the study were directed toward proteins that had been shown previously to be the targets for covalent binding of sulfonamide-reactive metabolites *in vitro* (36). Possibly, covalent binding of sulfonamide metabolites to microsomal proteins alter protein expression or function, thus leading to the induction of an immune response. While the antibodies formed may or may not play a role in the pathogenesis of sulfonamide-induced reactions, the fact that they are formed only in individuals who experience hypersensitivity reactions may be useful diagnostic information. However, as with the lymphocyte cytotoxicity assays described previously, the microsomal antibody assays currently are a research tool only.

Conclusions

The tools that are available for the diagnosis of sulfonamide-induced drug reactions remain limited at this time. Since the reactions themselves are clinically diverse and mechanistically multifactorial, no single diagnostic test alone will be sufficient in the evaluation of these patients. Information obtained through careful history-taking should help direct the physician toward diagnostic tests that may be useful. For patients who present with anaphylaxis or urticaria, beneficial information (i.e., the presence of sulfonamide-specific IgE antibodies) may be obtained from skin testing. In contrast, lymphocyte cytotoxicity or microsomal antibody assays

may yield more useful information for those patients who experience severe cutaneous reactions or the hypersensitivity-type syndrome, respectively. At this time, all of the diagnostic assays described remain research tools only. Hopefully, as we gather more knowledge about the mechanisms responsible for the various clinical reactions elicited by sulfonamides, these assays will soon move from the research laboratory into the clinical arena.

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