

Lymphocyte transformation studies in suspected hypersensitivity to trimethoprim-sulphamethoxazole

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Summary

In a group of patients with suspected and probable adverse skin reactions to trimethoprim-sulphamethoxazole, the lymphocyte transformation test (LTT) was positive in approximately 60% of subjects tested. Levels of stimulation in the majority of cases were low, probably reflecting either use of inappropriate stimulatory forms of the drug or lack of marked reactivity. Since there appeared to be a correlation between peak stimulatory index and duration of the rash, the latter explanation appeared more likely.

Introduction

Stevens–Johnson syndrome, a severe and dangerous hypersensitivity reaction to drugs has been frequently associated with the administration of long acting sulphonamides. The introduction of trimethoprim-sulphamethoxazole to clinical practice gave physicians a useful combination in which synergism between the constituents allowed the dosage requirement for the sulphonamide to be much less, resulting in apparently less risk of severe allergic reactions (Reeves *et al.*, 1969; Williams *et al.*, 1969; Reeves, 1971). Thus over the last 10 years or so few serious cutaneous adverse effects have been reported with this preparation. Nevertheless, since skin eruptions such as erythema multiforme, Stevens–Johnson syndrome, toxic epidermal necrolysis and exfoliative dermatitis are rare but significant complications of therapy (Azinge & Garrick, 1978; Wormser & Keusch, 1979), most physicians are extremely concerned when even mild cutaneous reactions occur, since it cannot be predicted if progression will occur to a more severe condition, either with continuation of therapy or re-introduction of the drug. Over the past few years, we have noted that referrals for assessment of reactions to trimethoprim-sulphamethoxazole are quite common, being second only to cases of suspected penicillin allergy. Since we have previously demonstrated the usefulness of

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the lymphocyte transformation test in the demonstration of immune reactivity to penicillins and to isoniazid (Warrington *et al.*, 1978; Warrington & Tse, 1979), we decided to investigate also its relevance to the diagnosis of suspected reactions to trimethoprim-sulphamethoxazole.

Methods

Subjects

The patients investigated were referred to the Section of Allergy and Clinical Immunology of the Health Sciences Centre, Winnipeg, because of suspected adverse reactions to trimethoprim-sulphamethoxazole. The characteristics of these patients are presented in Table 1. In most instances, this drug was thought to be responsible for the reaction because of its recent introduction. Where other antibiotics such as penicillins were administered at approximately the same time, skin tests were carried out to exclude immediate hypersensitivity to this group of drugs. In most instances, other drugs were being administered on a long term basis to the patients and were not discontinued at the time of the reaction. In all instances, it required the discontinuation of trimethoprim-sulphamethoxazole to cause regression of the skin eruption and other associated symptoms. In none of the patients was oral challenge with the drug undertaken. Normal controls in the study were volunteers without a history of drug allergy and not presently on medications.

Skin tests

In patients where a reaction to recent penicillin therapy was also possible, prick or intra-dermal skin tests were carried out with a major determinant of benzylpenicillin (benzylpenicilloyl-poly-L-lysine, Kremers-Urban Company, U.S.A.) at a concentration of $6 \times 10^{-5} \text{M}$ and a minor determinant mixture of benzylpenicillin or ampicillin, prepared by the method of Levine and Redmond (1969), used at a concentration of $2 \times 10^{-2} \text{M}$ (Warrington *et al.*, 1978).

Lymphocyte transformation test

This was undertaken as described previously (Warrington & Tse, 1979) using 0.2 ml microcultures with peripheral blood mononuclear cells at a cell density of 1×10^6 cells/ml. The trimethoprim-sulphamethoxazole was tested as a aqueous solution at concentrations of 0.1–500 $\mu\text{g/ml}$. Cells were incubated for 6 days at 37°C in 5% CO_2 and air and were labelled for 5 hr with ^3H -thymidine (specific activity 2 Ci/mmol, Amersham Corp., U.S.A.). Positive control cultures contained a 1:100 dilution of phytohaemagglutinin (PHA) (Difco Laboratories, U.S.A.). Labelled cells were harvested by automatic cell harvester and radioactivity assessed by liquid scintillation counting.

Results were expressed as the stimulatory index (S.I.), defined as the ratio of mean counts per min of all stimulated cultures at a single drug concentration to mean counts per min for non-stimulated (control) cultures. There was no significant difference between patient and control groups with regard to base line incorporation of ^3H -thymidine into control cells.

Table 1. Clinical characteristics of patients studied

Patient	Sex	Age (years)	Rash*	Pruritus	Symptoms	Duration (days)			Delay to LTT (months)	LTT
						Therapy	Rash	Other drugs		
1	F	18	E	+	None	14	21	Ampicillin	1	+
2	M	70	E/M	±	None	10	42	Ampicillin	5	+
3	F	38	E/P	+	Fever, nausea	5	3	Amoxacillin	3	+
4	F	25	A	+	None	30	2	Nalidixic acid tetracycline	1	-
5	F	12	U	-	Anorexia	14	2	Acetylsalicylic acid	1	±
6	M	36	E/P	-	None	7	5	Digoxin erythromycin	6	+
7	F	30	E	-	Fever, myalgia	1	1	None	18	-
8	F	21	E/M	+	Nausea, myalgia	14	3	None	5	-
9	F	24	E/M	+	Arthralgia	7	2	None	2	+

* E = erythematous; M = macular; P = papular; A = angioedema; U = urticaria.

Results

Controls

Nine normal controls without past history of drug hypersensitivity were assessed. Mean stimulatory indices for these individuals at the various drug concentrations tested ± 2 s.d. are given in Fig. 1.

Drug-sensitive patients

The characteristics of the nine tested patients are presented in Table 1. There were seven females and two males in the group studied. All had received the antibiotic for urinary tract infections. The majority of rashes associated with therapy were erythematous macular or maculopapular eruptions. Only two subjects suffered reactions more indicative of immediate hypersensitivity, i.e. urticaria or angioedema. Five of the nine patients suffered associated systemic symptoms such as fever, myalgia and arthralgia. Duration of therapy before onset of the rash varied from 1 to 30 days (mean 11.3 days) while the rash persisted for 1 to 42 days (mean 9 days). The lymphocyte stimulation tests were conducted from 5 days to 18 months after the drug eruption (mean 4.5 months). In six patients, other drugs were administered just prior to the initiation of trimethoprim-sulphamethoxazole or concomitantly with the antibiotic. In three such cases, the drug was a penicillin derivative. These patients were skin tested and found to be non-reactive to the penicillins. In a fourth case, tetracycline and nalidixic acid had been administered in this period. This patient was also studied with these drugs in the LTT and was non-responsive. A fifth patient received acetylsalicylic acid and has since taken this drug without problems. The final patient was taking digoxin on a long term basis and continued this drug and erythromycin while the rash regressed. Thus in most cases, except perhaps the fourth discussed here, trimethoprim-sulphamethoxazole was the most probable cause of the eruption.

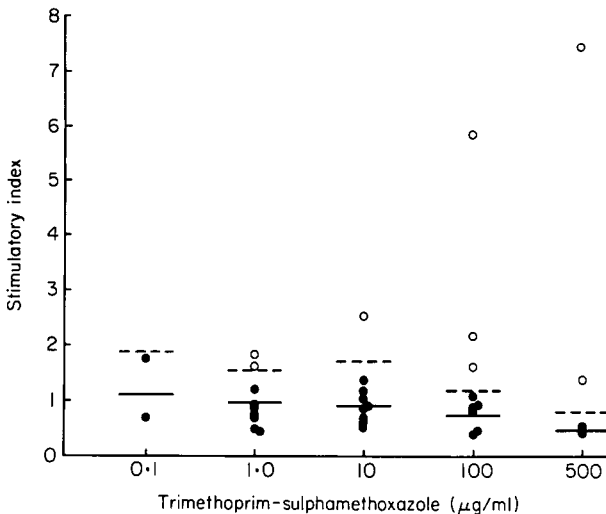


Fig. 1. Drug-induced stimulation of peripheral blood lymphocytes from nine patients with skin eruptions following the administration of trimethoprim-sulphamethoxazole. Results expressed as stimulatory indices. Horizontal lines indicate mean ± 2 s.d. from the mean for nine normal controls.

Lymphocyte transformation tests

As indicated in Fig. 1, 5 of 9 subjects tested showed a positive stimulatory index as defined by the LTT. In only one subject did the level of stimulation reach a marked level and here the response was 7.46 times control. The other individuals had S.I. of 1.4–2.52. Levels of incorporation of ^3H -thymidine in unstimulated cultures were similar in control and patient groups. All subjects tested had positive responses to PHA. For some subjects stimulation occurred with all concentrations of the drug used, while for the others, stimulation occurred only with a single, or at most two doses of the drug. It appears that a greater proportion of positive indices are found with higher concentrations of the drug (i.e. 100–500 $\mu\text{g/ml}$) (as we have shown previously for penicillins) despite some degree of probable non-specific suppression (since mean control S.I. at these concentrations are less than 1). This may indicate the need for spontaneous generation of stimulatory determinants in the cultures by cell metabolism, rather than the drug itself being the appropriate stimulant. There is no evidence for inherent mitogenicity of trimethoprim-sulphamethoxazole itself.

When the clinical characteristics of the patients studied were compared with the results of the LTT, there was no association noted between the nature of the rash, the presence or absence of pruritus, associated symptoms or duration of therapy and the presence or absence of lymphocyte stimulation. However, a correlation was found to exist between the duration of the rash and the peak stimulatory index ($r=0.9432$, $P<0.0005$), suggesting a relationship between subsequent *in vitro* lymphocyte reactivity and severity or chronicity of the eruption after discontinuation of the drug. Thus individuals in whom the rash persisted tended to have higher stimulatory indices.

Discussion

The combination antibiotic trimethoprim-sulphamethoxazole is used increasingly in clinical practice for the treatment of urinary tract infections, upper respiratory tract infections and similar diseases (Wormser & Keusch, 1979). This combination appears to have reduced the serious adverse effects associated with sulphonamides alone, such as Stevens–Johnson syndrome, toxic epidermal necrolysis and others, although these complications of therapy have been described with the drug combination (Azinge & Garrick, 1978; Wormser & Keusch, 1979). Perhaps because of this, physicians are understandably concerned when skin eruptions occur and will immediately discontinue the therapy. However, unlike penicillins, there is no proven diagnostic test that can be used to exclude true hypersensitivity, which means that individuals developing reactions while receiving trimethoprim-sulphamethoxazole are usually precluded from receiving this or other sulphonamide preparations in the future. Because we have previously found some value in using the LTT in demonstrating immune reactivity to penicillins and isoniazid in cases of suspected drug sensitivity, (Warrington *et al.*, 1978; Warrington & Tse, 1979) including some predictive value (Warrington, McPhillips-Feener & Rutherford, 1982) we have assessed the usefulness of the test in studying skin eruptions thought to be due to trimethoprim-sulphamethoxazole.

We have found that based upon the stimulatory indices using normal control peripheral blood lymphocytes exposed to the drug, approximately two-thirds of subjects investigated showed evidence of responsiveness to the drug *in vitro*. Of the three individuals that did not respond, one developed the eruption after only a single dose of drug, which was unusual for the group as a whole and in a second subject the reaction could have been to other drugs administered in the same period.

Despite the fact that overall, levels of stimulation were low, the fact that one individual showed quite marked reactivity suggested that an appropriate form of the drug was used, i.e. that a response to the preparation used was possible. In addition, although for most parameters examined, there was no association noted between clinical characteristics and the results of the lymphocyte studies, a definite relationship existed between duration (and perhaps severity) of the rash and peak S.I. This would suggest that *in vitro* reactivity does have some significance in terms of the clinical condition. Low levels of lymphocyte stimulation are a persistent problem in studies of drug hypersensitivity using the LTT and may reflect inappropriate forms of the drug used to stimulate the response, i.e. unknown drug metabolites or drug carrier conjugates may have to be generated *in vitro* before stimulation occurs. Perhaps this is why for most drugs studied it is necessary to use high concentrations of the drug in order to best elicit the response. At such levels, non-specific toxic effects may preclude the demonstration of a response. Certainly the degree of stimulation attained should at present be thought of as indicating an immune reactivity rather than pathogenetic mechanism.

A serious drawback with such a study is the failure to prove the value of the test by challenge. However, because of the potential serious side effects of re-administration and the lack of information regarding the validity of the test, it was felt that this procedure would be unethical unless the clinical importance of re-instituting therapy with trimethoprim-sulphamethoxazole outweighed the potential risks of challenge. In none of the subjects tested here was this criterion fulfilled. However, based upon the experience in this limited investigation, it does appear that further assessment of the value of the LTT in the diagnosis of drug hypersensitivity to trimethoprim-sulphamethoxazole is warranted.

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References

- AZINGE, N.O. & GARRICK, G.A. (1978) Stevens-Johnson syndrome (erythema multiforme) following ingestion of trimethoprim-sulphamethoxazole on two separate occasions in the same person. *Journal of Allergy and Clinical Immunology*, **62**, 125.
- LEVINE, B.B. & REDMOND, A.P. (1969) Minor haptenic determinant-specific reagins of penicillin hypersensitivity in man. *International Archives of Allergy and Applied Immunology*, **35**, 445.
- REEVES, D.S., FAIERS, M.C., PURSELL, R.E. & BRUMFITT, W. (1969) Trimethoprim-sulphamethoxazole: comparative study in urinary infection in hospital. *British Medical Journal*, **i**, 541.
- REEVES, D.S. (1971) Sulphamethoxazole-trimethoprim: the first two years. *Journal of Clinical Pathology*, **24**, 430.
- WARRINGTON, R.J., TSE, K.S., GORSKI, B.A., SCHWENK, R. & SEHON, A.H. (1978) Evaluation of isoniazid-associated hepatitis by immunological tests. *Clinical and Experimental Immunology*, **32**, 97.
- WARRINGTON, R.J., SIMONS, F.E.R., HO, H.W., GORSKI, B.A. & TSE, K.S. (1978) Diagnosis of penicillin-allergy by skin testing. The Manitoba experience. *Canadian Medical Association Journal*, **118**, 787.
- WARRINGTON, R.J. & TSE, K.S. (1979) Lymphocyte transformation studies in drug hypersensitivity. *Canadian Medical Association Journal*, **120**, 1089.
- WARRINGTON, R.J., MCPHILLIPS-FEENER, S. & RUTHERFORD, W.J. (1982) The predictive value of the lymphocyte transformation test in isoniazid-associated hepatitis. *Clinical Allergy*, **12**, 217.
- WILLIAMS, J.D., BURMFITT, W., COUDIE, A.P. & REEVES, D.S. (1969) The treatment of bacteriuria in pregnant women with sulphamethoxazole and trimethoprim. *Postgraduate Journal of Medicine*, **45**, Suppl., 71.
- WORMSER, G.P. & KEUSCH, G.T. (1979) Trimethoprim-sulphamethoxazole in the United States. *Annals of Internal Medicine*, **91**, 420.

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