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The predictive value of the lymphocyte transformation test in isoniazid-associated hepatitis

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Summary

Sixty-one patients receiving isoniazid (INH) for chemotherapy or chemoprophylaxis were assessed by the lymphocyte-transformation test (LTT) shortly after starting treatment. Thirty-eight per cent exhibited stimulation with INH, isonicotinic acid (INA) or human-serum albumin conjugates of these haptens. In the LTT-positive group, liver dysfunction subsequently developed in 58·8%, as compared to 22·7% in the LTT-negative group ($P < 0{\cdot}01$). The difference was not accountable on the basis of age, ethnic background, sex or chemotherapy vs chemoprophylaxis. Although there was an excess of alcohol abusers in the LTT-positive group, the probability of developing liver dysfunction amongst alcohol abusers in that group was twice as great as for LTT-negative alcohol abusers. The specificity of the LTT in predicting liver damage was 83–90% (depending upon the criteria used for determining positivity), while the sensitivity of the test was only 50%.

Introduction

Administration of the drug isoniazid (INH) is associated, in 10–20% of patients, with evidence of liver dysfunction (Bailey *et al.*, 1973; Beaudry *et al.*, 1974) which is mild in most cases and returns to normal with continuation of the drug. However, in approximately 1·0% of cases, there occurs the development of overt hepatitis (Garibaldi *et al.*, 1972; Maddrey & Boitnott, 1973; Kopanoff, Snider & Caras, 1978) which may be associated with a significant mortality (Black *et al.*, 1975).

It is possible that INH-induced liver damage results from the production of toxic metabolites such as acetylisoniazid and acetylhydrazine which may be formed more readily in fast acetylators (Mitchell *et al.*, 1976), either directly or through an immunologically-mediated reaction following their interaction with liver-specific antigens (Warrington & Olivier, 1979).

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We have previously demonstrated in a retrospective study of patients with INH-hepatitis that transformation of peripheral-blood lymphocytes occurs in response to the drug or drug-protein conjugate in approximately 95% of such subjects (Warrington *et al.*, 1978). However, the predictive value of the test in determining those patients at risk of developing hepatitis with the administration of the drug has not been assessed.

To examine this, we have assessed the association between a positive lymphocyte-transformation test (LTT) to INH and derivatives in the first 2–4 weeks of drug administration with the subsequent development of INH-associated liver damage.

Materials and methods

Subjects

Over a period of 18 months, from September 1977 to April 1979, an attempt was made to enroll all patients receiving chemotherapy or chemoprophylaxis with INH at this Centre, after explanation of the purpose of the study and after obtaining their voluntary consent and the permission of their personal physician. The subjects were contacted as soon as possible after being seen by their physician during the first 2–4 weeks of treatment and after obtaining consent, blood was drawn for the LTT. The patients were then followed over the course of their treatment with INH, with monthly (for the first 4 months) and later 3-monthly liver-function tests. Patients profiles were maintained on punched cards, recording age, sex, ethnic or racial background, concomitant drug therapy, history of alcohol abuse (including previous admissions for alcohol abuse, history of chemical abuses etc.), starting date and duration of therapy, in addition to monitoring abnormalities of liver function in the form of serum glutamic oxaloacetic transaminase (SGOT), bilirubin and alkaline phosphatase. The patient's personal physician was informed of the development of abnormal liver-function tests and the decision to continue or suspend therapy was made by him. Individuals in whom liver biopsy was not consistent with drug-induced liver damage or who were HbsAg positive were not included.

Lymphocyte stimulation

The LTT was carried out as described previously (Warrington *et al.*, 1978; Warrington & Tse, 1979), using the microculture technique. Briefly, human peripheral-blood lymphocytes were isolated from subjects on Ficoll-diatrizoate (LSM, Litton Bionetics, Maryland, U.S.A.). Cells were incubated in round-bottomed microwell culture-plates in sextuplet (Linbro; Flow Laboratories, Maryland, U.S.A.) at 37°C in 5·0% CO_2 in air, in RPMI 1640 (Gibco, Grand Island Biological Co., Grand Island, N.Y.) with 2 mM L-glutamine and gentamicin 50 μg/ml (Schering New Jersey, U.S.A.) supplemented with 10% heat-inactivated normal human AB serum. Lymphocyte cultures were stimulated with INH, isonicotinic acid (INA) and conjugates of INH and INA with human-serum albumin (HSA), at concentrations ranging between 10–500 μg/ml. The method of preparation of these conjugates has been reported (Tse, Vijay & Attalah, 1976). As a positive control, phytohaemaglutinin (PHA, Difco Laboratories, Michigan, U.S.A.) was added to cultures at a dilution of 1:100 of the stock solution. Cultures were incubated for 6 days and then proliferation was measured by incorporation of ^{3}H-thymidine (sp. act. 2 Ci/mmol, Amersham Co., Ontario, Canada), 1 μCi in 0·01 ml being added to each ml of culture and cells were incubated for a further

5 h, then harvested using a Skatron Automatic Cell harvester (Flow Laboratories). Radioactivity was assessed by liquid-scintillation counting in a Beckman LS 335 counter (Beckman Instruments, California, U.S.A.). The stimulatory index (SI) is defined as the ratio of counts from mitogen or antigen-stimulated cultures to non-stimulated cultures. Analysis of data was carried out by χ^2 or by Fisher's Exact test.

Results

Positive stimulation in the LTT was defined based upon our previously reported criteria (Warrington *et al.*, 1978; Warrington & Tse, 1979) by which an SI greater than 2 s.d. from the mean for control groups was considered to be positive (INH, $1{\cdot}13 \pm 0{\cdot}44$; INA, $1{\cdot}05 \pm 0{\cdot}34$; INH-HSA, $1{\cdot}17 \pm 0{\cdot}04$; INA-HSA, $1{\cdot}32 \pm 0{\cdot}35$).

There were sixty-one patients who co-operated in the study and for whom complete data was available. Liver damage occurred in 34·4% of these. This liver damage occurred within the first 3 months in 84% of cases and within the second 3 months of therapy for the remaining cases. Based upon the criteria above, 17 of 61 subjects (27·8%) demonstrated a positive LTT in response to INH or INA or the HSA conjugates. Liver damage developed in 58·8% of the LTT-positive group and in 22·7% of the LTT-negative group. As can be seen in Table 1, of the LTT-positive subjects with liver damage, 4 had elevated bilirubin and SGOT and 6 had only elevated SGOT. In the LTT-positive group, the majority (9 of 10 subjects) had only elevated SGOT.

Comparisons of LTT-positive and -negative groups

No significant difference existed in the age breakdown of two groups, being 41·5 years ($\pm 14{\cdot}8$) for the LTT-positive and 43 ($\pm 17{\cdot}8$) for the LTT-negative subjects. With regard to the sex breakdown (Table 2), there was a preponderance of males in the LTT-positive group, but this difference was not significant ($P > 0{\cdot}3$). No significant difference existed in the ethnic make-up of the two groups, in both approximately 47% of the subjects were Caucasians. There was no special weighting towards fast-acetylator groups i.e. ethnic Chinese. A difference existed in the numbers of alcohol abusers, in that 47% of the LTT-positive group had a history of alcohol abuse, as compared to 27·38% in the LTT-negative group. However this difference did not attain significance ($P = 0{\cdot}12$). No bias existed with regard to chemoprophylaxis *vs* chemotherapy and indeed there was not an increase in the rate of liver damage with chemotherapy (34% for chemotherapy and 33·3% for chemoprophylaxis, $P > 0{\cdot}6$).

Relationship between LTT and development of liver damage

If positive stimulation in the LTT was considered to be present when the SI was greater

Table 1. Liver damage in the LTT-positive and LTT-negative groups

	LTT+ve (%)	*P**	LTT−ve (%)
No. of subjects	17 (27·8)		44(72·2)
Liver damaged	10 (58·8)*	<0·01	10 (22·7)*
↑ bilirubin & SGOT	4		1
↑ SGOT	6		9

* Fisher's Exact Test

Table 2. Characteristics of LTT-positive and LTT-negative groups

	LTT+ve (%)		*P*	LTT−ve (%)	
Age (years)	41·5±14·8*			43±17·8*	
Ethnicity:					
Caucasians	8	(47)	>0·3†	21	(47·7)
Non-Caucasians	9			23	
Chemotherapy	12	(70·6)	>0·3	32	(72·7)
Chemoprophylasix	5			12	
Alcohol abusers	8	(47)	0·12	9	(27·3)

* Mean ± s.e.mean
† Fisher's Exact Test

than 2 s.d. from the mean for the control group, then in the LTT-positive group, 58·8% of subjects developed liver damage, as compared to 22·7% in the LTT-negative group. This difference was significant ($P<0·01$). If the criteria for positive LTT was made stricter i.e. SI > 3 s.d. from the mean for controls, then 71% of the smaller group of 14 patients developed liver damage, as compared to 21·2% in the LTT-negative group ($P<0·001$). When responses were broken down according to the stimulatory preparations used, in the liver-damaged group the LTT was positive with INH in 50% of subjects, with INA in 20%, with INH-HSA in 60% and in 70% with INA-HSA. In subjects without liver damage, the LTT was positive in 14·2% with INH and INA and 28·2% with INA-HSA while the LTT was negative with INH-HSA. Thus INH-HSA appeared to give the best discrmination. As occurred in our previous study (Warrington *et al.* 1978) several individuals responded to more than one form of the drug. Even within the LTT-positive group, a difference in the levels of maximal stimulation was apparent between those developing liver damage and those not developing damage (Table 3) suggesting that a relationship existed between the degree of immunological reactivity and the subsequent appearance of liver dysfunction. There were no significant differences with regard to the discrimination of the LTT in predicting liver damage in either Caucasians or non-Caucasians (62·5% of the former and 55·5% of the latter in the LTT-positive group developed liver damage as compared to 23·8% and 21·7% in the LTT-negative groups, $P=0·06$ and $P=0·7$ respectively). If all patients in the study were assessed, the probability of developing liver damage was

Table 3. Mean stimulatory indices (±s.e.mean) in LTT-positive group with and without liver damage

Stimulating preparation	INH	INA	INH-HSA	INA-HSA
Liver Damage +ve	4·88±1·97	3·68±1·82	4·87±1·65	4·28±1·39
	N.S.	N.S.	$P<0·05$*	N.S.
Liver Damage −ve	1·2±0·2	1·73±0·4	1·13±0·14	1·68±0·27

* Student's t-Test
N.S.-Difference not significant

0·5 in the LTT-positive group and 0·17 in the LTT-negative group ($P<0·01$). It is unlikely that this relationship was solely secondary to the increased proportion of alcohol abusers in the LTT-positive group. If only alcohol abusers in both groups were assessed, the probability of developing liver damage in the LTT-positive group was 0·875 as compared to 0·416 in the LTT-negative subjects. This difference was significant ($P=0·05$). The sensitivity of LTT in predicting liver damage was only 50% while the specificity was 83% when a SI greater than 2 s.d. from the mean for controls was considered positive and 90% when a stricter level of 3 s.d. from the mean was used.

Discussion

We have previously shown in a retrospective study that in patients developing hepatitis or persistent SGOT-elevations while receiving INH, the LTT was positive in 95% of cases in response to stimulation by INH, INA or HSA conjugates (Warrington *et al.*, 1978). However, the occurrence of lymphocyte transformation or stimulation with the drug or drug conjugates is not of itself indicative of the presence of INH-associated hepatitis, since INH will cause lymphocyte proliferation in cases of isoniazid-induced rash (Mathews, Pan & Wells, 1972; Warrington & Tse, 1979). We were therefore interesting in determining whether or not the occurrence of lymphocyte proliferation in response to these drug preparations was indicative only of a previous drug reaction involving the liver or the skin, or might be used as a predictive test to indicate subjects at risk of developing INH-associated hepatitis.

A total of sixty-one patients were assessed using the LTT within the first 4 weeks of therapy, of these 34·4% subsequently developed evidence of liver damage, 58·8% of the LTT-positive group and 22·7% of the LTT-negative group. Overall, five patients had elevated bilirubin and SGOT levels and fifteen had only elevated SGOT-levels. The increase in incidence of liver damage in the LTT-positive group was not explanable on the basis of differences in age, ethnic background or administration of chemotherapy *vs* chemoprophylaxis. There was an increased proportion of males and of alcohol abusers in the LTT-positive group. There is no evidence that the former may increase the incidence of liver damage during INH administration, but clearly the latter could influence the results. In the study however, a definite relationship was found to exist between the occurrence of a positive LTT and the development of subsequent liver damage even when only alcohol abusers were considered in the analysis. Indeed, for such alcohol abusers, the probability of developing liver damage was twice as high in the LTT-positive as in the LTT-negative group. Further support for the existence of a relationship between the LTT and the later development of liver damage comes from an examination of the levels of lymphocyte stimulation in the LTT-positive group, where the mean levels of stimulation for patients subsequently developing liver damage were significantly higher than those for subjects not developing liver damage. Nevertheless, while the specificity of the test was relatively good (83% increasing to 90% with stricter criteria for a positive LTT), the sensitivity was only 50%.

The lack of sensitivity requires some comment. In our retrospective study, in which the majority of subjects tested showed positive lymphocyte-stimulation, the patients were selected for having definite INH-associated hepatitis and those exposed to other potentially hepatotoxic factors were excluded. This was not possible in this prospective study because of low patient numbers. In addition, the patients in the earlier study had more severe liver damage overall than those tested here, since the number of subjects available to us necessitated inclusion of some individuals with only mild SGOT

abnormalities, where the association between drug-induced lymphocyte transformation and liver dyfunction may be less easily demonstrated (Warrington *et al.*, 1978). Also, it is possible that the low sensitivity may also reflect in some cases (1) use of inappropriate drug or drug metabolite-carrier conjugates, (2) alcohol-induced liver disease.

Nevertheless, the data in this study does show that, in about one half of patients tested, the occurrence of lymphocyte transformation in response to INH or related drug-preparations does predict the subsequent development of INH-associated liver damage, suggesting that such damage is at least associated with drug-specific immunological reactivity. In support of this, we have recently demonstrated the presence of drug and liver-specific cytotoxic lymphocytes in patients suffering from INH-associated hepatitis (Warrington & Olivier, 1979).

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