

## A clinical trial of levamisole in primary biliary cirrhosis

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### Summary

A small pilot study of levamisole in primary biliary cirrhosis failed to demonstrate any improvement in liver function and immunological abnormalities even in 'early' cases. This may be due to the fact that the defect in the immune system lies in its afferent arm. At present, levamisole, which is potentially toxic, cannot be recommended in conventional doses for the treatment of this disease.

### Introduction

The aetiology of primary biliary cirrhosis (PBC) remains unknown and while many immunological abnormalities have been observed, conventional immunosuppressive agents have not proved to be helpful (Carman and Gianseracusa, 1955; Howat *et al.*, 1966; Heathcote, Ross and Sherlock, 1976). The earliest lesion in PBC is infiltration of the intrahepatic biliary tree by mononuclear cells and granulomata (Rubin, Schaffner and Popper, 1965; Scheuer, 1967), suggesting cellular rather than humoral immunity might be of pathogenetic significance. Furthermore, since delayed hypersensitivity skin responses to common antigens and lymphocyte transformation to phytohaemagglutinin (PHA) are reported as defective in at least 50% of patients with PBC (Fox, Scheuer and Sherlock, 1973; Macsween *et al.*, 1973), it is possible that the disease is due to impaired cell-mediated immunity and might respond to the immunostimulant levamisole.

### Materials and methods

Informed consent was obtained from all 7 participants who exhibited the typical clinical features of PBC and had high titres of circulating antimitochondrial antibody.

The diagnosis was confirmed by liver biopsy in all cases.

At entry to the study immunoglobulin electrophoresis and autoantibody screening were performed by the routine hospital laboratory, and immune complexes measured by the PEG-CC method (Harkins and Brown, 1979). *In vitro* lymphocyte transformation to PHA and delayed hypersensitivity skin responses to tuberculin (PPD) were determined in each case. Skin sensitization with dinitrochlorobenzene (DNCB) was also carried out. All the skin testing agents used were of known efficacy, and the ability of the operator to perform the tests correctly was demonstrated by positive controls in normal subjects and some patients with chest diseases.

Levamisole (Janssen Pharmaceuticals) 150 mg orally on the same day each week was given for 6 months. The patients were seen weekly for 6 weeks and then monthly for a further 20 weeks. At each visit the patients were assessed clinically (note being taken of pruritus, jaundice, ascites, encephalopathy, liver and spleen size) and questioned about any possible side effects. A full blood count, liver function tests, urea and electrolytes were checked at each attendance. At the end of the study, the immunological investigations (except for the lymphocyte transformation to PHA, which was normal in all patients before treatment) were repeated.

### Results

Only 4 of the 7 patients (1, 4, 5, 7) completed the trial. Of the 3 who did not, one died of liver failure after 6 weeks, one died of variceal haemorrhage after 8 weeks (both had advanced disease) and one was withdrawn after 16 weeks because of carcinoma of the colon which had not metastasized to the liver and, in retrospect, was present before the trial.

Apart from the patient who died of liver failure, there was no alteration in liver function either

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clinically or biochemically (Table 1) during the trial. The humoral findings are shown in Table 2. Plasma IgA levels were within normal limits and did not change with treatment. IgG levels were generally raised, a finding described in cirrhosis from any cause, and remained unchanged during the trial. Only 3 of the 7 patients had raised IgM levels and there was no consistent change after levamisole. Antimitochondrial antibody was present in a titre of more than 1:400 in all patients and remained so throughout the study. Immune complexes were elevated in 6 of the 7 and during the trial became normal in only one case (of the 4 assessed).

TABLE 1. Mean values of standard liver function tests before and after levamisole in 7 patients with primary biliary cirrhosis

	Pre-trial	Post trial	P
Bilirubin ( $\mu\text{mol/litre}$ )	34	35	N.S.
Alkaline phosphatase (iu./litre)	629	667	N.S.
Albumin (g/litre)	38	39	N.S.
Serum glutamic pyruvic transaminase (iu./litre)	29	18	N.S.

Lymphocyte transformation to PHA was normal in all patients before treatment. However, they were anergic in their response to skin testing with common

antigens and no patient could be sensitized to DNCB (Table 3).

No patients experienced any significant side effect that could be attributed to levamisole, and in particular there were no blood dyscrasias. Indeed, 3 patients who entered the trial with platelet counts persistently less than  $100 \times 10^9/\text{litre}$  achieved normal counts within one week of starting therapy. The significance of this observation is uncertain.

## Discussion

The aetiology of PBC remains uncertain and while the condition may be immunologically mediated it does not respond favourably to conventional immunosuppressive measures (Carman and Gianseracusa, 1955; Howat *et al.*, 1969; Heathcote *et al.*, 1976). Although there are consistent alterations in humoral immunity (high plasma IgM levels (Feizi, 1968) and high titres of antimitochondrial antibody (Walker *et al.*, 1965) these may well be epiphenomena. Although recent interest has focused on the role of high molecular weight immune complexes (Thomas, Potter and Sherlock, 1977), the disease has other features to suggest defective cell-mediated immunity, for example granulomata in relation to bile ductules (Rubin *et al.*, 1965; Scheuer, 1967) and impaired delayed hypersensitivity skin response (Fox *et al.*, 1969). We postulated, therefore, that there might be impaired cell-mediated elimination of an unknown

TABLE 2. Plasma levels of immunoglobulins, antimitochondrial antibody and immune complexes before and after levamisole in 7 patients with primary biliary cirrhosis

Patient no.	Sex	IgA (g/litre)		IgG (g/litre)		IgM (g/litre)		AMA	Immune complexes	
		before	after	before	after	before	after		before	after
1	F	1.7	1.7	20.0	20.5	5	4.8	$\frac{1}{>400}$	30	28
2	F	4.7	—	19.0	—	6.0	—	$\frac{1}{>400}$	28	—
3	F	3.8	—	14.5	—	1.9	—	$\frac{1}{>400}$	34	—
4	F	1.1	1.4	10.0	10.0	2.1	1.5	$\frac{1}{>400}$	25	34
5	M	1.4	1.1	18.0	10.5	2.8	1.0	$\frac{1}{>400}$	26	7
6	F	2.6	—	15.5	—	1.1	—	$\frac{1}{>400}$	24	—
7	F	4.4	1.7	18.5	10.0	3.5	1.7	$\frac{1}{>400}$	1	24
Normal range		(1–3.5 g/litre)		(7–13 g/litre)		(0.5–2 g/litre)		—	(<24%)	

AMA: antimitochondrial antibody.

TABLE 3. Lymphocyte transformation to PHA and skin test results before and after levamisole in 7 patients with primary biliary cirrhosis

Patient no.	SK-SD		Mumps		Sensitization to <i>Candida</i>		PPD		DNCB	
	Before	After	Before	After	Before	After	Before	After	Before	After
1	0	0	+	+	++	++	+	+	0	0
2	0	N.D.	+	N.D.	++	N.D.	+	N.D.	0	N.D.
3	0	N.D.	+	N.D.	+	N.D.	0	N.D.	0	N.D.
4	+	+	+	0	++	++	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	+	N.D.	0	N.D.	+	N.D.	0	N.D.	0	N.D.
7	+	+	0	0	++	+	0	0	0	0

0 : no response

+ : weak response

++ : strong response

N.D. : not done

SK-SD : streptokinase-streptodornase

PPD : purified protein derivative (tuberculin)

DNCB : dinitrochlorobenzene

antigen and that levamisole, which stimulates cell-mediated immunity when this is defective (Anonymous, 1975), might prove valuable.

The patients studied all had the typical clinical and immunological features of PBC but there was no significant improvement in liver function during the period of study. This would be expected in those in whom severe liver damage had already occurred but two of our patients had 'early' disease and should have responded if our theory was correct. They did not do so.

Levamisole produced no improvement in cell-mediated immunity despite its use at a dose and for a duration effective in rheumatoid disease. Possibly a higher dose might have resulted in a measurable effect on the immune response but would be more likely to produce toxicity, particularly since the drug is metabolized by the liver. We observed no unusual effects of levamisole other than a rapid and striking improvement in the platelet count in 3 patients. Since thrombocytopenia is common in patients with cirrhosis and portal hypertension this observation may be worthy of further study.

In keeping with previous work we have found defective cell-mediated immunity as judged by skin testing in all our patients. However, in contradistinction to earlier studies, lymphocyte transformation to PHA was normal in all cases and we suggest that it is the afferent arm of the immunological response which is abnormal.

At present levamisole must join the list of immunoreactive drugs which have no proven value in the treatment of PBC.

#### Acknowledgments

We wish to thank Dr J. O. Hunter and Dr A. P. Dick for permission to study patients under their care, Mrs M. Shorthouse for

technical assistance and Dr D. Brown for immunological help and advice.

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