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Liver and biliary

Double blind controlled trial of d-penicillamine in patients with primary biliary cirrhosis

J NEUBERGER, E CHRISTENSEN, B PORTMANN, J CABALLERIA, J RODES, L RANEK, N TYGSTRUP, AND ROGER WILLIAMS

From the Liver Unit, King's College Hospital and Medical School, London, Hvidovre Hospital, Copenhagen, Denmark, Hospital Clinic i Provencial, Barcelona, Spain, and Rigshospitalet, Copenhagen, Denmark

SUMMARY One hundred and eighty nine patients with primary biliary cirrhosis were entered into a double blind, placebo controlled randomised trial starting in January 1978 to assess the therapeutic value of d-penicillamine 1200 mg daily. Eighteen of the 98 patients receiving d-penicillamine and 22 of the 91 placebo treated patients died during the study. Thirty six per cent of those on d-penicillamine and 8% of those on placebo were withdrawn from the study. No difference in overall survival was noted between the two groups of patients whether the results were analysed for the entire period of observation or only during the period in which the patients were receiving therapy. The mortality rate of those receiving d-penicillamine in histological stage I to II, however, was one third of that of the placebo group although this difference did not reach statistical significance. Using the occurrence rate ratio as the statistical method of analysis, no effect of d-penicillamine was noted on any clinical, biochemical or histological features examined, except the serum alanine aminotransferase activity which was greater in those on active treatment. In this trial we have been unable to establish any therapeutic benefit from the drug.

A number of therapeutic agents have been used in an attempt to control the underlying lesion in primary biliary cirrhosis. Corticosteroids have been associated with improvement of serum liver function tests,[1 2] but no controlled trial has been carried out and their bone thinning properties are likely to exacerbate the osteoporosis associated with the disease.[3] In one large multicentre placebo controlled trial azathioprine appeared to have some effect although the difference in survival did not reach statistical significance.[4] d-penicillamine was used initially because of its cupruretic effect[5] and its ability to remove immune complex-like activity from the serum of such patients.[6] It is now apparent, however, that the drug has other properties which may affect the course of primary biliary cirrhosis, such as its ability to interfere with collagen metabolism[7] and to reduce the number of circulating T lymphocytes.[8] After the publication of a preliminary study suggesting that survival was improved,[9] a number of controlled trials were set up, but the results to date are conflicting.[10–13] We report here the results of an international, double blind, placebo controlled trial set up in 1977.

Address for correspondence: Dr J Neuberger, Liver Unit, King's College Hospital and Medical School, Denmark Hill, London SE5 8RX.

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Methods

PATIENTS

Criteria for entry into the trial included a clinical and histological picture compatible with that of primary biliary cirrhosis, the liver biopsy being taken within six months before entry and a serum alkaline phosphatase more than twice the upper limit of normal in the absence of evidence of extrahepatic biliary obstruction. Those patients who had been taking azathioprine within six months were excluded from the study. Informed consent was obtained before entry into the study and approval was also given by the ethical committee in each hospital.

Patients were entered regardless of age or histological stage of the disease at diagnosis. Randomisation was performed separately for each Centre

(London, Barcelona, Copenhagen) and for each sex. Dosage was one tablet daily (containing either d-pencillamine 300 mg or lactose 50 mg), being increased by one tablet each fortnight until a maximum of four tablets daily were taken. In addition, all patients were given pyridoxine 20 mg daily. Patients were withdrawn from the trial if they developed signs or symptoms of possible side-effects from d-penicillamine, specifically thrombocytopenia (less than 60×10^9/l), neutropenia (less than 2×10^6/l) or proteinuria (greater than 2 g daily).

Clinical assessment was carried out at entry and at six monthly intervals. This included an estimation of the patient's well-being, as assessed by the incapacitation index described in detail elsewhere.[4] Blood was taken for estimation of standard liver function tests, serum immunoglobulins and autoantibodies. Each patient underwent a liver biopsy within six months of entry into the trial and at annual intervals. The biopsy specimens were assessed by a histopathologist (BP), without knowledge of treatment received or clinical condition of the patient.

STATISTICAL ANALYSIS

The death rate and the occurrence rate of clinical, serological, and histological features in patients who did not have those features at entry into the trial were analysed by the life table method using the log rank test for comparison of groups.[14] This method was used because it allows for incomplete follow up whether because of death, withdrawal, or loss from follow up. The occurrence rate ratio (or relative risk) was calculated as $(O_{PEN}/E_{PEN})/(O_{PLAC}/E_{PLAC})$ when O_{PEN} is the number of patients observed to develop a particular abnormal feature on d-penicillamine and E_{PEN} is the estimated number of patients in the d-penicillamine treated group expected to develop that feature. O_{PLAC} and E_{PLAC} are the corresponding values for the placebo treated group. The calculation of E_{PEN} and E_{PLAC} uses the temporal pattern of occurrence and assumes that the rate of occurrence is the same in the two groups;[4] hence a ratio of 0·5 means that the feature is half as likely to occur in the treated group as in the placebo group. Because this method requires that continuous variables, such as serum bilirubin, are analysed as discrete ones, we have arbitrarily chosen to define an occurrence if the value falls outside the 'abnormal' fifteenth percentile of the distribution of that variable at entry. Thus, with respect of serum bilirubin, an occurrence was deemed to occur when the serum concentration exceeded 90 μmol/l; and other limits are given in Table 1. Analyses were performed both for the total period of observation and up to the time of withdrawal.

Results

Between January 1978 and December 1982 when this analysis was performed, 189 patients had been entered into the trial. Of the total, 87 had been followed in London, 65 in Copenhagen and 37 in Barcelona. Analysis of the clinical, biochemical, serological, and histological data for patients in the d-penicillamine treated and placebo treated groups were similar at entry (Table 2). Nine patients receiving d-penicillamine were asymptomatic at the time of entry into the trial and eight in the placebo group.

In the d-penicillamine treated group 35 were withdrawn from treatment, because of rash (9),

Table 1 *Effect of d-penicillamine on the occurrence rate of clinical, biochemical and histological features after entry into the trial*

Variable	*Observed/expected ratio*		*Occurrence rates ratio (95% confidence limits)*	*p Value (2α)*
	d-penicillamine	*Placebo*		
Clinical features				
Pruritus/cholestyramine	16/13·03	26/28·97	1·37 (0·77–2·42)	0·78
Ascites/diuretics	14/15·74	21/19·26	0·82 (0·45–1·50)	0·52
GI bleeding	4/4·81	8/7·19	0·75 (0·26–2·14)	0·59
Incapacitation index >25	22/22·74	24/23·26	0·94 (0·54–1·64)	0·83
Biochemistry				
Bilirubin >90 μmol/l	7/10·30	14/10·70	0·52 (0·21–1·26)	0·15
Albumin <30 g/l	13/14·80	16/14·20	0·78 (0·38–1·61)	0·50
IgM >6·9 g/l	15/11·14	14/17·86	1·72 (0·91–3·25)	0·09
Histology				
Stage III/IV	21/17·75	13/16·26	1·48 (0·77–2·86)	0·24
Stage IV	10/11·35	13/16·23	0·79 (0·35–1·76)	0·56

Bilirubin 1 μmol/l = 0·02 mg/dl. Albumin 1 g/l = 0·1 g/dl.

Table 2 *Clinical, median biochemical, serological and histological details of patients in the placebo and d-penicillamine groups at entry into the trial*

	Placebo	*d-penicillamine*
Cases (no)	91	98
Males (%)	7	9
Median duration of history (years)	1·6	1·8
Frequency of clinical findings		
Pruritus	63%	59%
Jaundice	46%	37%
Xanthoma	22%	17%
GI bleeding	17%	22%
Ascites	7%	13%
Liver function tests (normal range)		
Bilirubin (3–20 μmol/l)	21·0	23·9
Alkaline phosphatase (3–85 IU/l)	504	502
Alanine aminotransferase (7–40 IU/l)	72	80
Cholesterol (3–8·3 mmol/l)	6·5	6·3
Albumin (35–50 g/l)	35·2	35·4
IgM (0·5–2 g/l)	3·5	3·6
Mitochondrial antibodies %	88	85
Liver histology		
Stage I	15%	12%
Stage II	37%	40%
Stage III	21%	24%
Stage IV	27%	24%

Bilirubin 1 μmol/l = 0·02 mg/dl. Albumin 1 g/l = 0·1 g/dl.

proteinuria (5), thrombocytopenia (4), gastrointestinal upset (4), rash and arthralgia (3), non-compliance (3) and one each for leucopenia, asthma, pemphigoid, ageusia, psychosis, and palpitations. The final patient was withdrawn when a liver transplant was considered to be indicated. Seven patients were withdrawn from the placebo treated group on account of headaches (2), gastrointestinal upset (1), proteinuria (1), neurological complications (1), non-compliance (1) and liver transplantation (1). Significantly more patients withdrew from the treatment group than the control group ($p<0·0001$) (Table 3).

No significant difference in overall survival between patients in the placebo and d-penicillamine treated groups was observed (Figure); this was independent of whether the total observation period or only the time up to withdrawal was used for calculation of the curves. The p values are calculated after stratification according to each centre, even though the effect of d-penicillamine on survival was not significantly different in the three centres. Separation of the patients into early (histological stage I/II) and late (stage III/IV) showed an apparent beneficial effect on survival when d-penicillamine was used in the early stages of the disease, the mortality rate being one third of that seen in the placebo treated group. The difference, however, did not reach statistical significance ($p=0·15$). None of the patients asymptomatic at entry died during the course of the trial. In patients with late stage disease the death rates were nearly identical in the two groups ($p=0·99$). Survival in those patients who were able to tolerate the full dose of d-penicillamine was similar to survival in the control group.

The occurrence rate for gastrointestinal bleeding, pruritus (or the need for cholestyramine) and ascites (or use of diuretics) was similar in both groups. The only difference with respect to liver function tests, biochemical, and serological findings was that the use of d-penicillamine was associated with a significant increase in serum alanine aminotransferase, above 136 IU/l. There was no difference in the rate of histological progression as assessed from the

Table 3 *Reason for withdrawal from the trial and median time of withdrawal after institution of treatment*

	d-penicillamine			*Placebo*		
Cause	*No*	*Time*	*(Range)*	*No*	*Time*	*(Range)*
Rash	9	4 months	(13 days–7 months)			
Proteinuria	5	8 months	(3 days–24 months)	1	7 months	
Thrombocytopenia	4	11 months	(1–19 months)			
Rash and arthralgia	3	3 months	(1–17 months)			
Gastrointestinal upset	4	11 months	(2 weeks–18 months)	1	7 months	
Leucopenia	1	24 months				
Asthma	1	3½ months				
Pemphigoid	1	12 months				
Ageusia	1	8 months				
Psychosis	1	1 week				
Palpitations	1	3 months				
Liver transplantation	1	11 months		1	7 months	
Non-compliance	3	1 month	(3 days–8 months)	1	2 weeks	
Neurological complications				1	2 months	
Headaches				2	3½ months	(3–4 months)

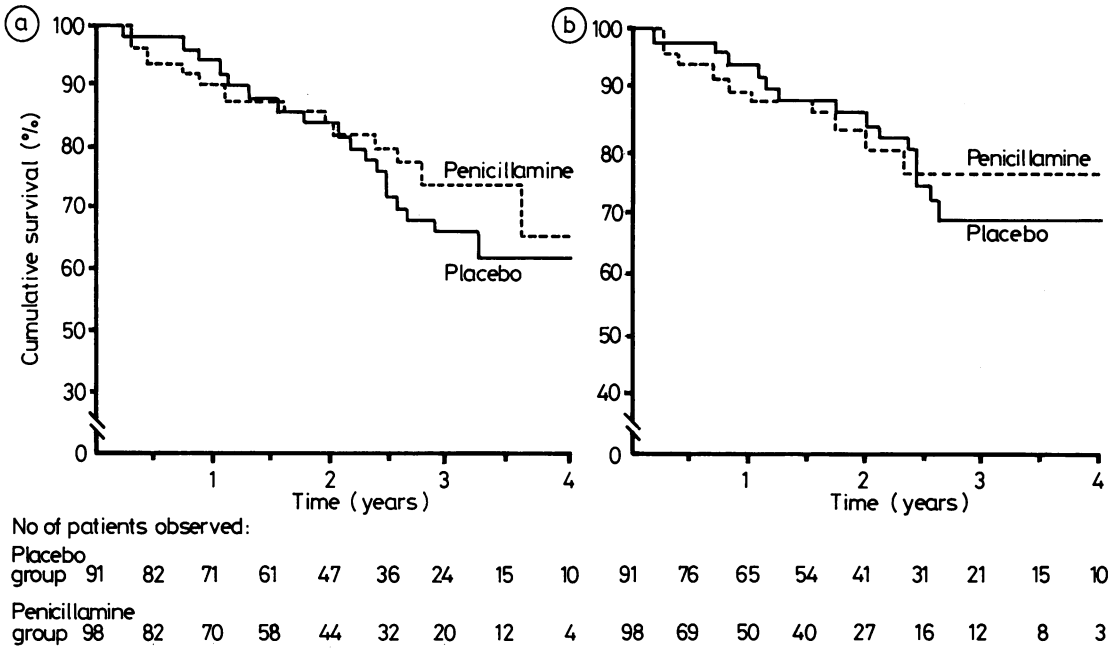

Figure *Cumulative survival for patients with primary biliary cirrhosis receiving either placebo or d-penicillamine for (a) total period of observation (ORR=0·91, p=0·78) and (b) during period of receiving therapy (ORR=0·98, p=0·96). Number of patients observed in each group represents the number of patients alive at that time.*

serial biopsies obtained and d-penicillamine did not appear to prevent progression of the disease from early to late histological stages (Table 1). Analysis of the serological and histological findings in the two groups based on the time until withdrawal gave results similar to those in Table 1, which are based on the entire period of observation.

Forty patients had died by the time of analysis (Table 4). Of the 18 who died in the d-penicillamine treated group, 16 were associated with liver disease and the remaining deaths were because of pre-existing asthma and cardiovascular disease. Of the 22 deaths in the placebo treated group, 18 were associated with liver disease and the remainder were because of cardiovascular disease, renal failure, bronchial carcinoma, and following a fractured femur. There was no significant difference in the number of deaths or distribution of the main causes of death between the two groups.

Table 4 *Causes of death in patients entered into the trial*

	Placebo	*d-penicillamine*
Related to liver disease		
Liver failure	10	10
Liver failure and GI bleeding	4	4
GI bleeding	2	1
Bacterial peritonitis	1	–
Hepatocellular carcinoma	1	1
Unrelated to liver disease		
Renal failure	1	–
Asthma	–	1
Bronchial cancer	1	–
Fractured femur	1	–
Cardiovascular disease	1	1
TOTAL	22	18

Discussion

The use of the occurrence rate ratio to analyse the effect of treatment with d-penicillamine on the survival, clinical, and serological variables was chosen because it is a more effective discriminant of the population trend than measuring the percentage increase or decrease of the variables with time. For example, an increase in serum bilirubin from 8–12 μmol/l will show the same percentage increase in levels as that from 80 to 120 μmol/l. In contrast, with the use of the occurrence rate ratio, only the latter example will be recorded as an 'event' or occurrence. Yet clearly the implications on the natural history of the disease are quite different: levels of serum bilirubin in the normal range are indicative of a good prognosis[15] whereas the rising

levels indicate a poor prognosis and advancing disease.[16] The other advantage of this method of analysis is that it allows for incomplete follow-up of patients for any cause. This method of analysis, however, does require continuous variables to be treated as discrete ones. The arbitrary use of the fifteenth percentile allows a reasonable number of patients to have 'events' recorded. Comparable findings, however, were obtained when different cut-off figures were used. In this report we have analysed results for patients up to the time of withdrawal as well as patients followed during the entire period of the trial. The former analysis may be biased if the patients who were withdrawn from the trial, usually because of drug-related side effects, differ in prognosis from those not withdrawn. Analysis based on the intention to treat may be more valid although the effect of a short and not maintained course of d-penicillamine on the natural history of a disease lasting many years may be questionable. Nonetheless, both methods of analysis gave very similar results.

The various trials of d-penicillamine reported to date differ both in trial design and in dosage used. The largest study is that from the Mayo Clinic[13] in which patients were given placebo or d-penicillamine 1000 mg. There was an associated improvement in survival at all stages of the disease and progression of liver histology from early to late stages was reduced. One death was attributed to side-effects from the drug. In contrast, Epstein *et al*[9] used d-penicillamine at a daily dose of 600 mg and found no effect on those treated at early stages, although there was a significant improvement in those treated in histological stages III and IV after 18 months of treatment. In a study carried out in Boston[15] d-penicillamine was not associated with improvement of any variable measured; indeed, those on active treatment had a slightly worse prognosis. In Newcastle, Bassendine and her colleagues[11] were able to show that, at a daily dose of 1000 mg, there was an improvement in serum transaminase and immunoglobulins, while at lower doses (250 mg daily) the improvement was only temporary. Survival was not affected compared with placebo in either group. In Sheffield it was found that in doses up to 875 mg daily there was improvement in liver histology, although no effect on survival was found.[12]

To what extent these differences in results are because of selection of patients or different population studied is not clear. It is now becoming apparent that the syndrome of primary biliary cirrhosis covers a wide spectrum of disease activity. It may be possible to identify a subgroup of such patients whose disease activity may be controlled by long term therapy with d-penicillamine, although such identification was not possible in our trial. The mode of action of the drug in primary biliary cirrhosis, if any, is not clear. Although introduced because of its ability to reduce liver copper content and reduce the levels of circulating immune complexes. It is probable that the liver copper is not hepatotoxic in primary biliary cirrhosis:[17] any improvement noted in patients on treatment with d-penicillamine may not be associated with a reduction in hepatic copper.[18] Reductions in levels of circulating immune complexes is another probability, but the importance of immune complexes, and indeed their existence in primary biliary cirrhosis, has recently been questioned.[19] d-penicillamine and its use requires that the patient attends frequently for blood and urine monitoring. Side effects both in this trial and in the trials reported occurred in up to 30% of patients.

The maximum period of follow up of patients in the trial is short in comparison with the natural history of the disease. Accordingly, the confidence limits of our results are not very narrow and it is still possible that significant effects on survival will be noted, perhaps in subgroups, when follow-up is longer. For these reasons, it is intended to continue the study although no new patients will be added.

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