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THE EFFECT OF PENICILLAMINE ON VITAMIN B_6 FUNCTION IN MAN

PAUL C. RUMSBY and DAVID M. SHEPHERD*
Department of Pharmacology and Therapeutics, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY, U.K.

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Abstract—The erythrocyte alanine aminotransferase (Ala-AT) activity, measured *in vitro*, of rheumatoid arthritis patients under treatment with penicillamine was markedly stimulated by the addition of excess pyridoxal-5′-phosphate (PLP). This indicates that penicillamine can produce a deficiency of vitamin B_6 possibly by reacting chemically with the coenzyme PLP or by inhibiting PLP synthesis. The deficiency, though demonstrable by biochemical tests, was not accompanied by clinical signs of vitamin B_6 lack, and it would not justify the administration of a pyridoxine supplement to penicillamine-treated patients except in those of low nutritional status. In addition to its effect on the coenzyme PLP penicillamine also decreased the concentration of the erythrocyte Ala-AT apoenzyme.

The first clinical applications of penicillamine (β,β-dimethylcysteine) were in the treatment of Wilson's disease [1] and cystinuria [2]. Subsequently, penicillamine was found to be of value in the treatment of rheumatoid arthritis [3], and, following extensive clinical trials [4], it has since been widely used for this condition. As penicillamine contains an asymmetric carbon atom, it exists in D-, L- and DL-forms. The D-isomer, being much less toxic than the L-isomer [5], is the one which is normally used therapeutically.

As the L-isomer was known to have an anti-vitamin B_6 effect in the rat [6, 7], it became important to ascertain whether the D-penicillamine in clinical use had an anti-vitamin B_6 effect in man. In due course reports appeared of D-penicillamine-induced vitamin B_6 deficiency which was readily reversible by the administration of pyridoxine to the affected patients [8–10]. Usually there were no clinically obvious symptoms of vitamin B_6 deficiency; however, the underlying deficiency was revealed by the greatly increased urinary excretion of xanthurenic acid which occurred when the penicillamine-treated patients were given a large oral dose of tryptophan (the Tryptophan Load Test). In the studies now reported, vitamin B_6 deficiency in rheumatoid arthritis patients treated with D-penicillamine was assessed by measuring the percentage stimulation of their erythrocyte alanine aminotransferase [Ala-AT; L-alanine: 2-oxoglutarate aminotransferase (EC 2.6.1.2)] when an excess of pyridoxal-5′-phosphate (PLP) was added *in vitro*.

MATERIALS AND METHODS

Blood samples were obtained from patients with rheumatoid arthritis who were receiving penicillamine and attending the Rheumatology Clinic at Dundee Royal Infirmary. The treatment commenced with a low dose (125 mg/day), and this was raised by 125 mg/day at intervals of 1–2 months, usually to 750 mg/day or occasionally to a maximum of 1 g/day. Approximately 33% of the patients were taken off the drug owing to side-effects such as severe rashes, mouth ulcers or low platelet counts. These side-effects were not considered to be due to impairment of vitamin B_6 function. The vitamin B_6 status of many of the patients was assessed several times during their treatment with penicillamine. Most of these patients were also receiving other anti-inflammatory drugs such as prednisolone, indomethacin or naproxen.

A group of patients attending either the Rheumatology Clinic at Dundee Royal Infirmary or the Medical Outpatients Clinic, Ninewells Hospital, Dundee, served as controls. These fell into two categories: (a) rheumatoid arthritis patients receiving anti-inflammatory drugs other than penicillamine, including gold, prednisolone and indomethacin; and (b) patients receiving either no medication or drugs considered unlikely to have an effect on vitamin B_6 status.

Blood was collected in tubes containing lithium heparin, and assayed for erythrocyte Ala-AT activity within 72 hr by the method of Woodring and Storvik [11]. The enzyme activity, determined with and without the stimulation of added PLP *in vitro*, was expressed as μmole pyruvate formed per millilitre of packed red blood cells per hour. The stimulation was calculated from the formula:

$$\% \text{ Stimulation} = \frac{(\text{Ala-AT activity with PLP}) - (\text{Ala-AT activity without PLP})}{\text{Ala-AT activity without PLP}} \times 100.$$

* Author to whom all correspondence should be addressed.

Patients in whom this stimulation exceeded 25% were considered to be vitamin B_6 deficient [12, 13].

Differences in the number of patients having Ala-AT stimulation greater than 25% in the treated and control groups, respectively, were assessed for significance by χ^2 analysis. Differences in enzyme activities were assessed by Student's t test.

RESULTS

There was no significant difference in the mean age of the patients in the control and penicillamine-treated groups (Table 1).

Of 375 observations on 144 penicillamine-treated patients 66 (17.6%) showed erythrocyte Ala-AT stimulation greater than 25%, while of the 79 controls only 6 (7.6%) showed stimulation exceeding 25%; the difference between these percentages was significant ($P < 0.05$).

In those penicillamine-treated patients who showed greater than 25% stimulation of erythrocyte Ala-AT activity by excess PLP *in vitro*, the stimulated activity, instead of rising to that of the controls, remained significantly less (Table 2; $P < 0.001$).

In penicillamine-treated patients the incidence of Ala-AT stimulation exceeding 25% was greatest at the lowest dose level (125 mg daily) and also at the highest dose level (1 g daily), (Fig. 1).

DISCUSSION

The above results indicate that penicillamine can produce a deficiency of vitamin B_6 in man. This conclusion, based on the criterion of greater than 25% stimulation of erythrocyte Ala-AT activity by the addition of excess PLP *in vitro*, is in accord with that reached by previous workers on the basis of the increased urinary xanthurenic acid excretion which follows the tryptophan load test in penicillamine-treated patients [8, 9]. As the deficiency is detectable only by biochemical tests, and as it is not accompanied by overt clinical symptoms of vitamin B_6 deficiency, supplementation of penicillamine-treated patients' diet with pyridoxine would not be required except in those of low nutritional status; Jaffe [9] was also of this opinion.

It is interesting to consider separately those penicillamine-treated subjects whose erythrocyte Ala-AT showed greater than 25% stimulation on addition of excess PLP *in vitro*. Their unstimulated Ala-AT activity was significantly lower than that of the controls (Table 2, column 3) as would be

Table 1. Details of patients studied

Patients	No. of patients	No. of observations	Age (years) Mean ± S.E.M.	Range
Controls (79)				
Arthritic	43	43		
Non-arthritic	36	36		
Male	34	34	57.3 ± 3.7	25 - 82
Female	45	45		
Penicillamine-treated (144)				
Male	41	103		
Female	103	275	54.8 ± 1.1	21 - 79

Table 2. Comparison of stimulated and unstimulated Ala-AT activities in controls and in those penicillamine-treated patients who showed greater than 25% stimulation in response to excess PLP added *in vitro*

Subjects	Number	Ala-AT activity (μmol pyruvate/ml packed RBC/hr) Mean ± S.E.M. Unstimulated	Stimulated	P
Controls	79	2.93 ± 0.19	2.99 ± 0.19	< 0.001
Penicillamine-treated showing stimulation > 25%	66	1.13 ± 0.07	1.61 ± 0.10	

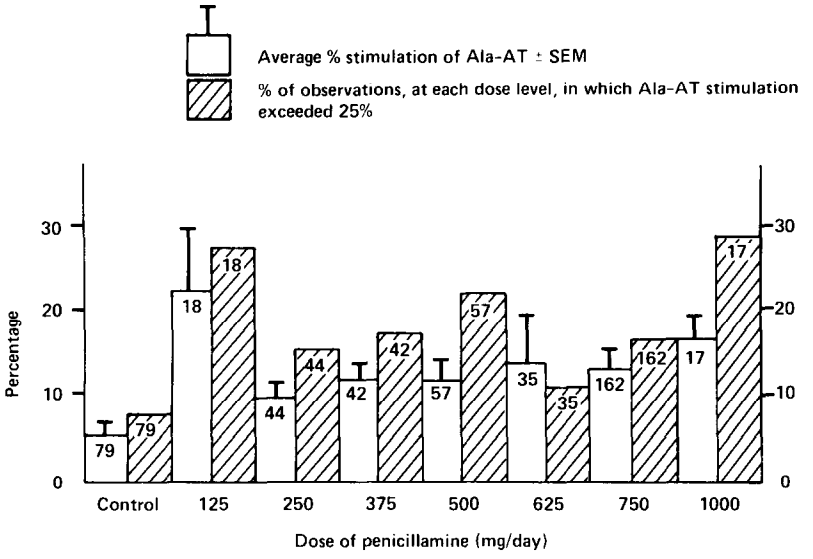

Fig. 1. Effect of penicillamine dose on erythrocyte Ala-AT in control patients and those receiving penicillamine. Figures at the top of each column are the number of observations at that particular dose level.

expected if the coenzyme is deficient. Thus, when the excess PLP is added to saturate the apoenzyme, the stimulated Ala-AT activities of the penicillamine-treated patients should then reach the same level as that of the controls. However, the stimulated Ala-AT activity of these patients remained significantly less than that of the controls (Table 2, Column 4). This indicates that a reduction has occurred in the amount of apoenzyme in the erythrocytes of the treated group.

Simultaneous decrease of coenzyme and apoenzyme levels has been reported for the PLP-dependent enzyme cysteine sulphinic acid decarboxylase in the liver of vitamin B_6-deficient rats [14, 15]. There is evidence that vitamin B_6 regulates the formation of apoenzymes for which it is the coenzyme [16, 17]; it also stabilises them [18, 19]. Thus, vitamin B_6 deficiency could lead to decreased tissue levels of these apoenzymes. Moreover, in rat small intestine there is an enzyme which specifically inactivates the apoenzymes of PLP-dependent enzymes [20], and the tissue concentration of this enzyme is known to be increased in penicillamine-treated rats [21]. Thus, the decreased amount of Ala-AT apoenzyme which we have found in the erythrocytes of penicillamine-treated patients could be due to decreased formation or increased degradation of the apoenzyme, or to a combination of both factors.

In comparison with controls there was a greater incidence of Ala-AT stimulations exceeding 25% in patients receiving penicillamine at the lowest dose level of 125 mg/day and also at the highest dose level of 1 g/day (Fig. 1); however, there were relatively small numbers of observations at these two dose levels. The stimulation was less marked at intermediate dose levels. These results suggest that a vitamin B_6 deficiency may be produced in the early stage of treatment and that some recovery then takes place, the deficiency reappearing only in cases where the dose is subsequently raised to a high level.

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