Pyridoxine-responsive Anemia: Influence of Tryptophan on Pyridoxine Responsiveness

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This study presents evidence that the indolic amino acid, tryptophan, critically influences pyridoxine responsiveness in a patient with pyridoxine-responsive anemia. Continuing observations for 18 yr on this now 54-yr-old white man have shown responsiveness to oral crude liver extract and to pyridoxine. In addition, remissions of 8-mo duration followed oral administration of fractions of liver extract in doses of 2 mg daily. These nonprotein fractions contained acidic substances characterized as indoles. From 1959 to 1970, oral pyridoxine, in daily doses of 12.5 mg for 5 days, predictably produced prompt reticulocytosis, fall in serum iron content, and rise in hematocrit to nearly normal levels. Such responses to pyridoxine consistently lasted less than 2 mo. More recently, relative refractoriness to this dose of pyridoxine has developed. However, responsiveness has been restored by oral administration of L-tryptophan, 750 mg daily, an amount alone insufficient to maintain the hematocrit at optimal levels. These observations indicate relationships of tryptophan and pyridoxine in erythropoiesis that have not been recognized previously. Addition of L-tryptophan to the therapy of certain pyridoxine-responsive and other sideroblastic anemias might be considered.

IN THIS STUDY, evidence is presented that indicates that pyridoxine and the amino acid, tryptophan, are involved in erythropoiesis in a manner not previously recognized. The basis of this evidence consists of 18 yr of continuous observation of a now 54-yr-old white man who presented initially with severe hypochromic, microcytic anemia and hyperferremia, for which extensive studies revealed no primary cause. The bone marrow was characterized by erythroid hyperplasia and maturation arrest with a predominance of basophilic normoblasts. Megaloblastic changes were not apparent. Previously reported studies of this patient have indicated that, although refractory to therapy with vitamin B₁₂ and folic acid and to multivitamin preparations containing niacin, riboflavin and thiamin, his anemia was dramatically responsive to oral crude liver extract (Valentine). Moreover, prolonged remissions followed oral administration of very small amounts of fractions derived from this extract. These derivatives contained nonprotein substances of small molecular weight that had chemical and physical characteristics of indolic acids. Remission of greater than 8-mo duration followed oral administration of such fractions in doses of 2 mg daily for 7 days.

The anemia in this patient is also responsive to pyridoxine. Between 1959 and 1970, response to oral pyridoxine HCl in doses of 12.5 mg daily for 5 days...
was completely predictable. These remissions, however, were quite short, i.e., consistently under 2-mo duration. In this report, recent observations are presented that indicate the development of relative refractoriness to this dose of pyridoxine and subsequent restoration of responsiveness following administration of L-tryptophan. These observations suggest that the responsiveness to pyridoxine in this patient depends on the availability of tryptophan or an indolic derivative thereof.

MATERIALS AND METHODS

Routine hematologic investigations were carried out according to established methods. Serum iron concentrations (performed in the Hematology Laboratory, Cleveland Metropolitan General Hospital, through the courtesy of Dr. John W. Harris) were determined by the method of Schade et al. Scored tablets of pyridoxine HCl 25 mg (kindly supplied by Merck, Sharp & Dohme, West Point, Pa.) were split in half for 12.5-mg doses. Doses of 0.25 mg were prepared by appropriate dilution of a stock solution of powdered pyridoxine HCl (obtained from Nutritional Biochemicals Company, Cleveland, Ohio), 1.0 mg/ml in 0.05 N HCl, and were stored at -20°C. Purity and concentration of such dilutions were verified by determining their ultraviolet absorption spectrum in 0.1 N HCl. (The ultraviolet absorption spectrum of pyridoxine HCl in 0.1 N HCl shows a sharp peak of maximum absorption at 291 m.μ, at which wavelength the extinction coefficient of a 1% solution in a 1.0 cm light path is 422.) L-Tryptophan (obtained from Nutritional Biochemicals Company, Cleveland, Ohio), 250 mg, was prepared in gelatin capsules (size No. 00) for oral administration.

RESULTS

Figure 1 shows a typical response to pyridoxine HCl given in the oral dose of 12.5 mg daily for 5 days. There was a prompt reticulocyte response with a peak
Fig. 2. Predictability of response to pyridoxine HCl, 12.5 mg daily by mouth for 5 days, during a period of 17 mo. Hematocrit was maintained by these intermittent courses of pyridoxine while testing the activity of various fractions of liver. None of such fractions tested in this period showed significant activity, but minimal activity in some may have influenced the period of relapse, e.g., from Sept. 28, 1962 to Dec. 19, 1962.

of 8.6% on the seventh day after initiation of therapy. Prompt rises in the hematocrit from 31% to 43.5% and in the hemoglobin concentration from 9 to 13.4 g/100 ml occurred. The serum iron concentration fell promptly from 282 to 78 μg/100 ml in 11 days. The duration of this remission, measured as the interval from the initiation of therapy to the point at which falls in the hematocrit and hemoglobin concentration become apparent, was 24 days.

Predictability of the response to this amount of pyridoxine is shown in Fig. 2. During the 17-mo period from July 1, 1962 to December 15, 1963, seven such remissions occurred, each lasting from 18 to 49 days. During this period, as in others before and since, intermittent administration of this dose of pyridoxine served to maintain the patient's hematologic status during testing of various fractions of liver in attempts to identify further the indolic substance previously shown to produce prolonged remissions of 8 mo or greater. Reproducible conditions for the extraction of such fractions, although under continuing study, remain elusive.

A failure in August 1970, of the previously predictable responsiveness to pyridoxine is shown in Fig. 3. At this time, the “standard” course of pyridoxine HCl, 12.5 mg daily for 5 days, failed to produce the predictable rises in hematocrit and hemoglobin concentration. There was no clue in his clinical presentation at this time to explain the development of this refractoriness. There were no signs of infection, and no discernible changes in dietary or other habits were elicited.

With failure of the previously predictable response to pyridoxine, L-tryptophan, 250 mg three times daily by mouth, was given for 4 wk. With this, prompt hematologic improvement occurred; in 2 wk the hematocrit rose from 37% to 40%, and the hemoglobin rose from 10.8 to 12.75 g/100 ml. Nearly normal values were then maintained for 3 wk after the tryptophan was discontinued.

With subsequent hematologic relapse, evidenced by continuing falls in both hematocrit and hemoglobin concentration (Fig. 3, day 105), L-tryptophan was
again administered in the same oral dose of 250 mg three times daily. The previously observed rises in hematocrit and hemoglobin concentration within 2 wk did not occur. However, addition of pyridoxine HCl, 0.25 mg daily by mouth for another 2 wk, was accompanied by a response that was maintained for 2 wk after discontinuing both tryptophan and pyridoxine.

In Fig. 4, the course is shown immediately following that of Fig. 3 and indicates changes in the hematocrit and serum iron concentration associated with
another suboptimal response to pyridoxine that was enhanced by tryptophan. The administration of pyridoxine HCl, 12.5 mg daily for 5 days, was followed in 3 wk by suboptimal rise in the hematocrit to 40% and by a fall in the serum iron concentration from 201 to only 183 μg/100 ml. Administration of l-tryptophan, 250 mg three times daily for 2 wk, was again followed by a further rise in the hematocrit to 45% and a further fall in serum iron concentration to 87 μg/100 ml. Once again, increase in the serum iron concentration occurred as relapse approached 6 wk after discontinuing the tryptophan.

At this point (Fig. 4, day 88), “optimal” responsiveness to pyridoxine alone appears to have been restored. Administration of the same dose of pyridoxine alone was followed by a sharp fall in the serum iron concentration to 39 μg/100 ml and a rise in the hematocrit that was maintained at nearly normal levels for 71 days. With relapse again (Fig. 4, day 182), the same dose of pyridoxine alone was again followed by a remission of 70-days duration.

DISCUSSION

These recent patterns of response indicate that erythropoiesis in this patient with pyridoxine-responsive anemia appears dependent on the availability of tryptophan, as well as pyridoxine. Previous evidence for involvement of tryptophan in the pathogenesis of his anemia consisted of a suboptimal and transient rise in the hematocrit following a single oral dose of 4 g of l-tryptophan in a load test. Additional evidence consisted of a remission of 8-mo duration produced when a 2-wk course of pyridoxine HCl, 0.25 mg daily, was given 3 mo after beginning a course of l-tryptophan, 750 mg daily, which was continued for a total of 7 mo. During the first 3 mo of this 7-mo course, tryptophan alone did not maintain the hematocrit at optimal levels (Fig. 5 in reference 4). Similarly prolonged remissions have occurred following the administration of small amounts of indolic fractions of liver and following the interaction of other indolic liver fractions, alone inactive, with pyridoxine (Figs. 8 and 9 in reference 4). However, the apparent dependence of pyridoxine responsiveness on the availability of these indoles, including tryptophan, was not appreciated at the time these observations were made.

Continuing administration of intermittent courses of pyridoxine alone without tryptophan or without intervening responses to active derivatives of liver eventually resulted in a state of refractoriness. The refractory state to pyridoxine HCl, 12.5 mg daily for 5 days, observed in August 1970 (Fig. 3), occurred 3½ yr after the previous course of tryptophan. It was the third successive such course of pyridoxine following a 3-mo response to a suboptimally active liver fraction given 14 mo previously.

Further, the data indicate that the restoration of pyridoxine responsiveness apparently depended on prolonged or repeated courses of tryptophan and pyridoxine. Figures 3 and 4 show that three courses of therapy involving both substances were required before optimal responsiveness to pyridoxine alone was restored. In addition, the apparent requirement of pyridoxine for responsiveness to l-tryptophan at the 750 mg daily dose level is demonstrated. In Fig. 3, prompt effectiveness of l-tryptophan within 2 wk is shown when administered
17 days after the course of pyridoxine HCl, 12.5 mg daily for 5 days. However, 100 days after the course of pyridoxine, administration of tryptophan produced no such immediate response; and additional pyridoxine, 0.25 mg daily for 2 wk, was required for hematologic effectiveness. The effect of this uncommonly small amount of pyridoxine is curious, especially since it is but a small fraction of the 2–3 mg that is estimated to be in the normal daily diet of the adult. Response to this small dose of pyridoxine had been observed previously when given in conjunction with tryptophan and with an indolic fraction of liver that alone was inactive (Figs. 5 and 8 in reference 4); however, when given subsequently, remote from either tryptophan or liver fractions, this dose of pyridoxine was ineffective. Therefore, the present response shown in Fig. 3 appears to be a restoration of responsiveness to the 0.25 mg daily dose of pyridoxine by the administration of tryptophan and suggests a very sensitive dependency of erythropoiesis on vitamin B₆ that in turn depends on the availability of tryptophan or of an indolic derivative thereof. Throughout the 18 yr of observation of this patient, there have been no other signs of vitamin B₆ lack including dermatitis and peripheral neuritis, and the tryptophan load test was normal.

Although admittedly inconclusive at this time, these observations suggest that the interaction of pyridoxine and tryptophan in some way conditions subsequent responsiveness to pyridoxine alone. Possibly this might occur through the formation of an intermediate that is retained endogenously for long periods for subsequent interaction with pyridoxine alone. The observation that three tryptophan-pyridoxine interactions were required before restoration of optimal pyridoxine responsiveness suggests that the effects of these interactions were cumulative. The possible relationship of such a proposed intermediate to indolic fractions of liver previously shown to have erythropoietic activity in this patient is speculative. The prolonged period required to develop pyridoxine refractoriness following response to such a liver fraction might support such a relationship.

The nature of the relationships among tryptophan, pyridoxine, and indolic liver fractions in the erythropoiesis of this patient needs further clarification. Available evidence indicates that neither the niacin nor the serotonin pathway of tryptophan metabolism is involved. Possible involvement in globin synthesis and possible similarities to protein synthetic effects of indoleacetic acid in plant tissue have been discussed in a previous publication. Although this patient presents clinically as a “typical” sideroblastic form of pyridoxine-responsive anemia, his patterns of therapeutic response are quite unusual among such patients. Nevertheless, a practical consideration presented by the observations in this patient is whether these relationships might be involved in other patients with primary sideroblastic anemia, particularly in those with partial responsiveness to pyridoxine and in those who, although initially responsive, become refractory to pyridoxine, as well as in those who are refractory to all forms of therapy. The clues presented in this patient indicate that the addition of a trial of L-tryptophan, along with pyridoxine, to the therapeutic armamentarium for such patients might be considered.
REFERENCES


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