PYRIDOXINE DEFICIENT DIET AND DESOXYPYRIDOXINE IN THE THERAPY OF LYMPHOSARCOMA AND ACUTE LEUKEMIA IN MAN

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Evidence from animal experimentation indicates that pyridoxine, a component of the vitamin B complex, is an essential factor for the maintenance and function of hematopoietic tissue. Severe anemia has been induced in dogs and swine on a pyridoxine deficient diet, and the integrity of lymphoid tissue in rats has also been demonstrated to be dependent upon this vitamin. The significance of pyridoxine in human nutrition is unknown inasmuch as no known deficiency state involving this vitamin alone has been described. Recently, Stoerk reported that lymphosarcoma transplants failed to grow in mice maintained on a pyridoxine deficient diet; he further noted that established transplants of lymphosarcoma regressed when mice were placed on a pyridoxine deficient diet together with the specific vitamin antagonist, desoxypyridoxine.

In the experiments reported in this paper, an attempt was made to induce a pyridoxine deficiency in man to determine any possible therapeutic effects in lymphosarcoma and acute leukemia. Although no significant clinical improvement resulted from the experimental therapeutic regimen, the results are of interest in that they indicate certain fundamental differences in the utilization of pyridoxine in lower animals and man.

MATERIALS AND METHODS

Six patients were placed on a pyridoxine deficient diet and given desoxypyridoxine. Three patients had disseminated lymphosarcoma and 3 had acute leukemia. It was planned to keep the subjects on this diet for fourteen days. However, in several instances the caloric intake was limited so severely by the unpalatability of the diet that the regimen was of necessity discontinued earlier. The basic constituents of the diet were: (a) vitamin-free casein, (b) gelatin, (c) sugar, (d) cornstarch, (e) unenriched cream of wheat, (f) butter, (g) artificial flavoring. The patients were also allowed carbonated drinks such as ginger ale and Coca-cola ad libitum, tea and coffee without cream or milk, and one serving of peaches or one apple per day. The dietician, working with this very limited number of foodstuffs, prepared cookies and puddings to provide some variety. However, all of the food had a "chalky" consistency and flavor which rapidly became extremely distasteful to the patients. Therefore, although an adequate amount of protein, carbohydrate and fat, as well as an adequate number of calories were provided, in only two of the patients was the diet completely consumed. In addition to the above, the

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This work was supported by a grant from the American Cancer Society, on recommendation of the Committee on Growth of the Vocational Research Council.

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patients were given appropriate doses of thiamine, nicotinic acid, riboflavin and ascorbic acid as individual vitamins.

Desoxypyridoxine* was given to the first 2 patients studied in doses of 2.5 mg. per Kg. per day in three equally divided doses by mouth. Due to toxic manifestations which will be described later, the dose was progressively decreased to approximately 1.5 mg. per Kg. per day in the other patients of the series.

Disturbance of tryptophane metabolism as manifested by xanthurenic acid and kynurenine excretion in the urine has been reported in experimental animals on a pyridoxine deficient diet.8-10 Daily 2.4 hour urine specimens were collected on 2 of the patients and xanthurenic acid determinations were made according to the technic described by Porter.5

Case Reports

Case 1. D. T. This 6 year old white boy had developed painless swelling at the base of his neck with associated fever, cough and 'wheezing' respirations beginning four weeks before hospital entry. Aside from recurrent asthma and eczema his past history was negative. Examination revealed slight fever (100.2 F.), wheezing but unlabored respirations, hoarseness, generalized lymphadenopathy, massive in left cervical and axillary regions and probably retroperitoneal as well, with splenomegaly. Positive x-ray findings were limited to the chest where a widened mediastinum and a large anterior mediastinal mass were noted. Blood studies revealed normochromic anemia, a normal leukocyte count and differential, and normal sternal bone marrow. Cervical node biopsy disclosed lymphocytic lymphosarcoma.

Following a four day course of methyl bis (d-chloroethyl) amine totalling 0.4 mg. per Kg., the temperature fell to normal, appetite and weight increased, hoarseness disappeared and visible nodes regressed. Within the ensuing week, lymphadenopathy recurred. Shortly thereafter the patient was placed on a pyridoxine free diet for four days together with the pyridoxine analogue, desoxypyridoxine (2 mg. per Kg. per day), and 1.0 Gm. L-tryptophane — both in three equal daily doses. L-tryptophane was given to accentuate any possible disturbance in the metabolism of this amino acid. Throughout this period, appetite decreased markedly; concomitant weight loss of 0.5 Kg., and continued enlargement of nodes with tracheal compression were noted. Of interest was the fact that the peripheral blood values remained unchanged and no xanthurenic acid was excreted in any of the four 2.4 hour urine specimens during these four days. Lymph node response to a second course of methyl bis (d-chloroethyl) amine administered at this time was minimal and the patient was discharged to receive radiotherapy at an institution nearer his home after a lapse of two weeks. Although therapeutic response to irradiation appeared to have been excellent, one month later he developed recurrent epistaxis, rectal bleeding and abdominal pain and died at home. The total duration of his illness had been approximately four months.

Case 2. R. C. This 52 year old, white housewife was hospitalized for progressive painless enlargement of all superficial lymph nodes over a six months period with increasing nasal obstruction and hearing loss, as well as pain in the right hip. Pertinent physical findings were some loss of hearing, hyperplasia of pharyngeal lymphoid tissue in addition to generalized lymphadenopathy (including retroperitoneal area probably) hepato-splenomegaly and fever. Blood corpuscle counts and differential, urinalysis, liver function tests and x-rays of chest and sinuses were normal. Fasting serum sugar was 58 mg. per cent. An inguinal node biopsy revealed reticulum-cell sarcoma.

The patient received a single injection of 36.4 mg. (0.44 mg. per Kg.) of methyl bis (d-chloroethyl) amine through technical error, the only objective toxic effects of which were marked nausea and vomiting, minimal diarrhea without occult blood in stools and profound leukopenia. Because of poor and transient therapeutic response she was placed on a pyridoxine free diet with added desoxypyridoxine at three hour intervals during the day, totalling 15 mg. per Kg. daily. After a total of 1.5 Gm. of desoxypyridoxine had been given she developed persistent nausea and suddenly lost consciousness, became cyanotic and exhibited mass movements of extremities of a convulsive character. Following spontaneous

* Generously supplied by Dr. D. F. Robertson, Merck Institute for Therapeutic Research.
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recovery in about two minutes. 100 mg of pyridoxine was given parenterally. The following day residual sequelae were noted in the form of diaphoresis, nausea and some amnesia for recent events, and the dose of desoxypyridoxine was lowered to 2.5 mg per Kg daily in three equal doses. During a fourteen day period on the deficient diet, weight loss, mental confusion and a 50 per cent reduction in lymph node size occurred. Repeated blood counts showed no abnormalities or changes, and a fasting blood sugar of 51 mg per cent did not differ significantly from the control value. On the hypothesis that additive effects might be obtained, a four day course of methyl bis (8-chloroethyl) amine was then given, totaling 0.4 mg per Kg; there was further regression of adenopathy and some weight gain. The patient died suddenly within two weeks following hospital discharge at another institution. No necropsy was performed. The total duration of her illness had been nine months.

Case 1. J. S. This 52 year old white salesman entered the hospital with painless enlargement of the glands in his neck over a seven week period, associated latterly with dysphagia, weakness and weight loss. Examination disclosed generalized lymphadenopathy, particularly in the neck, hepatosplenomegaly and signs of fluid at the right lung base. Peripheral blood showed no anemia but a leukocytosis with a relative increase in lymphocytes some of which were immature forms. X-rays of the chest revealed a right paratracheal shadow and confirmed the clinical impression of right pleural effusion. Biopsy of an axillary node disclosed reticulum-cell lymphosarcoma. Therapeutic response to a four day course of methyl bis (8-chloroethyl) amine totaling 0.4 mg per Kg was poor in that adenopathy remained stationary, right pleural fluid increased in amount and an exhausting nonproductive cough developed. Chest fluid revealed histologic changes compatible with lymphosarcoma at this time. The patient was then placed on a pyridoxine deficient diet with added desoxypyridoxine totalling 2.5 mg per Kg daily in three divided doses. During this period anorexia and cachexia became marked, and he exhibited mental dullness, somnolence, increasing cough and edema of both legs and low grade afternoon fever. Some observers felt cervical lymph nodes became softer and smaller during this two weeks. The blood picture remained unchanged throughout, and no xanthurenic acid excretion was detected in 24 hour urine specimens. Forty-eight hours after resuming a regular diet the patient died suddenly. His illness had lasted about three months. Autopsy performed nine hours postmortem showed gross evidence of widely disseminated invasive lymphosarcoma involving liver, spleen, heart, lungs, stomach and kidney as well as nodes in the mediastinum and abdomen. Microscopic examination confirmed these findings and in addition showed similar changes in prostate, bone marrow and thyroid gland. The nervous system appeared grossly and microscopically normal. Splenic sections were of interest in that scattered fields showed multinucleated lymphoid cells, fragmentation of nuclear material and minimal necrosis about lymphoid cell clusters. Dr. H. C. Stoerk who kindly reviewed these sections stated these latter sections bore some resemblance to changes seen in lymphoid tumors of pyridoxine deficient animals. However, in the main, comparison of the morphology of the tumor from autopsy material showed no significant variations from that seen in the pretreatment biopsy sections.

Case 2. G. U. This 8 year old white boy had a 3 week history of pharyngitis, cervical adenopathy and fever and showed hypertrophic gums, enlargement of the liver and all superficial nodes. Blood studies including sternal marrow aspiration were compatible with the diagnosis of acute monocytic leukemia. After fourteen days on a pyridoxine free diet with added vitamin antagonist, physical and hematologic findings remained unchanged. During the ensuing month his condition degenerated rapidly with continued fever, weight loss and hemorrhagic phenomena and he died at home in the ninth week of his illness.

Case 3. E. U. Blood studies confirmed the clinical impression of acute lymphatic leukemia in this 3 year old white male with symptoms of one month duration and fever, extensive purpura and marked hepatosplenomegaly on physical examination. During 13 days of pyridoxine deficient diet with added antagonist the WBC dropped from 6100 to 1650, blast forms from 70 to 25 per cent and RBC from 3.39 to 1.54 millions with parallel hemoglobin changes. Ten days later the patient died in coma. No autopsy was performed. Total duration of his disease was two months.

Case 4. J. K. This 4 year old white male with symptoms of six weeks duration showed pallor, generalized adenopathy, hepatosplenomegaly and petechiae. Blood studies showed a high percentage of blasts and a diagnosis of acute leukemia was made. On the fifth day of a pyridoxine deficient diet with added desoxypyridoxine totalling 25 mg per Kg., he had two generalized convulsive seizures in rapid succession following which the desoxypyridoxine was reduced to 1.5 mg per Kg. Because the child be-
came irrational for several hours twenty four hours later, the latter medication was discontinued, but the
dietary regimen was prolonged until death eight days after its institution. During this period a marked
and progressive leukocytosis with an increase in blasts from 90 to 100 per cent and a fall in RBC and
Hgb. values were noted. Death occurred at the end of this period in coma after an illness totalling two
months duration. No autopsy was performed.

Discussion

In 2 of the 3 patients with lymphosarcoma there was evidence of moderate re-
gression in the size of the lymph nodes. It is unwarranted to ascribe this change
specifically to a pyridoxine deficiency, for Stoerk has shown that in rats exposed to
adverse dietary conditions there is an approximately linear relationship between the
body weight deficit and the thymic weight deficit.4 On the reasonable assumptions
that (a) an analogous situation pertains to man and (b) that the decrease in the
weight of the thymus is an expression of generalized lymphoid atrophy, it is prob-
able that the observed changes in the lymphadenopathy of our patients were co-
incident with the nonspecific malnutrition. This conclusion is further strengthened
by the fact that there were no demonstrable morphologic evidences of specific
cellular change attributable to pyridoxine deficiency in the tumor of the patient
who came to necropsy.

The marked depression of hematopoietic function described in pyridoxine de-
ficient experimental animals was not clearly demonstrated in the patients of this
study. Admittedly it is difficult to assess this particular point in patients with
acute leukemia and widely disseminated lymphosarcoma. Since all of the cases had
anemia of varying degrees of severity at the onset of the dietary regimen, it was
impossible to determine the effect of the diet and desoxypyridoxine on erythropoie-
sis. It is to be noted, however, that leukopenia, lymphocytopenia, and thrombo-
cytopenia did not occur during the period of observation except in one case of acute
leukemia. In this patient there was no significant alteration of the hemogram and
the development of leukopenia is entirely compatible with the natural course of
the disease.

The two episodes of central nervous system excitation seen in our patients were
probably an expression of acute pyridoxine deficiency induced by the large doses
of the pyridoxine antagonist, desoxypyridoxine. Mushett et al.,11 have reported
convulsions in experimental animals given large doses of desoxypyridoxine, and
Wintrobe and his associates have described convulsive seizures in pyridoxine de-
ficient swine which closely resemble grand mal epilepsy.12 The signs and symptoms
seen in our patients were also similar to this cerebral dysrhythmia.

Kynurenine and xanthurenic acid are abnormal metabolites of tryptophane
which are excreted in the urine of animals which are deficient in pyridoxine.8–10
Xanthurenic acid excretion in man has not been noted10 and this has suggested that
tryptophane degradation varies in different species. To our knowledge, such a
rigorous pyridoxine deficient diet has not previously been employed in the studies
of xanthurenic acid excretion in humans. Inasmuch as 2 of the patients failed to
excrete xanthurenic acid while on the diet and while receiving desoxypyridoxine,
additional circumstantial evidence is provided that tryptophane is not metabolized
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in the same way in all species; however, the possibility that the patients were not
deficient in pyridoxine cannot be excluded.

The criteria of a pyridoxine deficiency in experimental animals include depression
of hemopoiesis, atrophy of lymphoid tissue, demyelinating lesions of the central
and peripheral nervous system, and disturbance of tryptophane metabolism.
Applying these criteria to the patients reported here would lead to the conclusion
that (a) either a pyridoxine deficiency had not been induced or (b) pyridoxine is
not essential in human nutrition. A final possibility is that the manifestations of
vitamin B6 deficiency in man are entirely different from those observed in animals
and that they were unrecognized in the patients of this study. It is impossible to
state with assurance which of the above possibilities pertained in these experi-
ments. In mice, Stoerk has observed lymphoid regression within five days after
the onset of desoxyppyridoxine and a pyridoxine restricted diet.13 This would in-
dicate that there are not large stores of the vitamin in the body. Wintrobe and his
associates, on the other hand, noted that in swine on a pyridoxine deficient diet
two to seven weeks passed before there were unmistakable signs of the specific
vitamin deficiency.12 Since there was clear evidence of nonspecific malnutrition in
our patients within fourteen days, a more protracted period of dietary experimenta-
tion was not justifiable.

It may be stated unequivocally that, under the conditions of the experiment,
there was no evidence that restriction of the pyridoxine intake together with de-
soxypyridoxine had any therapeutic value. In the 3 patients with lymphosarcoma,
a course of nitrogen mustard (methyl bis (beta chloroethyl) amine) was given fol-
lowing the completion of the experimental dietary period to determine whether a
greater response would be produced by the chemotherapeutic agent. There was no
significantly greater regression of the tumor masses observed following the post-
pyridoxine deficiency nitrogen mustard therapy than had occurred with previous
chemotherapy.

SUMMARY AND CONCLUSIONS

Three patients with disseminated lymphosarcoma and 3 cases of acute leukemia
were placed on a pyridoxine deficient diet together with desoxyppyridoxine for
periods of four to fourteen days. Although there was evidence of malnutrition in
the form of weight loss and weakness, none of the signs of specific pyridoxine de-
ficiency described in animals was observed in the human. There was no unequivocal
evidence of depression of hemopoiesis, no significant atrophy of lymphoid tissue,
no signs of demyelinization of nerves, and no abnormality of tryptophane metab-
olism determined by urinary xanthurenic acid excretion. The possibility that the
dietary restriction of pyridoxine was too brief for the development of a deficiency
syndrome was considered, but it was pointed out that the rigors of the procedure
were too great to justify more protracted periods of observation.

Two patients had acute toxic manifestations following the administration of
large doses of desoxyppyridoxine. These were characterized by transient epilepti-
form convulsions. There were no sequelae and no recurrence of the symptoms when
the dose of the drug was reduced.
There was no evidence that the restriction of pyridoxine in the diet together with desoxypyridoxine for periods up to two weeks had any therapeutic effect in lymphosarcoma or acute leukemia. Also, no potentiation of the cytotoxic effect of nitrogen mustard was observed in the patients with lymphosarcoma when chemotherapy was given after the completion of the pyridoxine deficient regimen. It is to be emphasized that this does not absolutely exclude the possibility that pyridoxine deficiency may adversely affect lymphosarcoma in man. The short duration of the experiment and the well known refractoriness to any form of therapy of the tumors in these patients are factors which may have militated against a satisfactory outcome of the regimen.

REFERENCES


13 Stork, H. C.: Personal communication.
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