Studies on Tryptophan Metabolism in Patients with Lymphoma

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SUMMARY

The 24-hr urinary excretion of certain metabolites of tryptophan was measured in 22 patients with Hodgkin’s disease and 18 patients with lymphosarcoma, following the p.o. administration of 2 g L-tryptophan. An assay of hepatic tryptophan pyrrolase activity was done on 15 patients with Hodgkin’s disease and 15 patients with lymphosarcoma, and an assay of hepatic kynureninase was done on 11 and 7 patients with Hodgkin’s disease and lymphosarcoma, respectively. There was increased urinary excretion of kynurenine in 9, of 3-hydroxykynurenine in 16, of kynurenic acid in 4, of xanthurenic acid in 4, and of acetylkynurenine in 3 patients with Hodgkin’s disease. Similarly, there was an increase in urinary excretion of kynurenine in 5, 3-hydroxykynurenine in 7, kynurenic acid in 1, xanthurenic acid in 1, and acetylkynurenine in 5 patients with lymphosarcoma. The increased urinary tryptophan metabolite excretion was more common and more marked in patients with Stage III and IV Hodgkin’s disease, particularly the symptomatic Group B patients. However, this correlation could not be made in the patients with lymphosarcoma because of the limited number of patients with Stage I and II disease. There was no correlation between tryptophan pyrrolase activity and either the level of urinary excretion of tryptophan metabolites or the stage of the disease. On the other hand, patients with low hepatic kynureninase activity tended to excrete increased quantities of these tryptophan metabolites. Depressed kynureninase activity and increased excretion of tryptophan metabolites were more marked in advanced Stage III and IV Hodgkin’s disease.

INTRODUCTION

One of the metabolic pathways of tryptophan proceeds through a series of enzymatic degradations into kynurenine and several other intermediary metabolites to nicotinic acid (Chart I). A key enzyme in this metabolic sequence is tryptophan pyrrolase, a hepatic heme-containing enzyme that catalyzes the oxidation of tryptophan to formylkynurenine. Several of the enzymes in this pathway utilize pyridoxal phosphate as a cofactor, and one of these, kynureninase, which catalyzes the conversion of kynurenine to anthranilic acid and 3-hydroxykynurenine to 3-hydroxyanthranilic acid, would appear to play a key role.

Increased urinary excretion of some of the metabolites of the tryptophan-niacin pathway, with or without prior tryptophan loading, has been observed in many clinical states such as pregnancy, rheumatoid arthritis, scleroderma, porphyria, and several neoplastic diseases (8). In the latter, frequent increased excretion of these metabolites was reported in cancer of the bladder (2) and of the breast (6), in lymphomas, particularly Hodgkin’s disease (1, 3), and to a lesser extent, in other neoplasms. The etiology and significance of these abnormalities is not yet clear.

Altman and Greengard (1) noted a linear correlation between the level of hepatic tryptophan pyrrolase activity and the urinary excretion of kynurenine in patients with a variety of diseases, most of whom had rheumatoid arthritis. Tryptophan pyrrolase is inducible by tryptophan or cortisol, and it is conceivable that the neoplasms and other disease processes can produce increased activity of this enzyme resulting in increased urinary excretion of some of the metabolites of tryptophan. In addition, there might be decreased activity of some of the enzymes involved in the tryptophan-niacin pathway, leading to a slowing of some of the catalytic processes, with resulting accumulation of certain metabolites. Chabner et al. (3) reported an increased excretion of at least 1 of 3 tryptophan metabolites measured (kynurenine, 3-hydroxykynurenine, and xanthurenic acid) in 14 of 21 patients with untreated Hodgkin’s disease. Serum pyridoxal phosphate was measured in 14 of the patients, 8 of whom had subnormal levels of the vitamin. The increased urinary tryptophan metabolite excretion and low serum pyridoxal phosphate were most frequent in the symptomatic patients and in those with more extensive disease.

In the present investigation, an attempt was made to correlate hepatic tryptophan pyrrolase and kynureninase activities in patients with Hodgkin’s disease or other lymphomas with the urinary excretion of some of the tryptophan metabolites after a 2-g dose of L-tryptophan.

MATERIALS AND METHODS

Forty patients with histologically established untreated lymphoma (22 with Hodgkin’s disease, 7 with diffuse histiocytic lymphoma, 7 with diffuse, poorly differentiated lymphoma, and 4 with nodular, poorly differentiated lymphocytic lymphoma) were admitted to Roswell Park Memorial Institute for either closed staging of their disease (investigation by clinical and radiological procedures in-
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Chart 1. Abbreviated pathway for metabolic conversion of tryptophan to nicotinic acid.

including lymphangiography, needle bone marrow, and liver biopsies) or staging by laparotomy. The patients were subjected, after an informed consent was obtained, to the following investigative procedures.

L-tryptophan, 2 g, blended in ginger ale, was given at 9:00 a.m. This was followed by a 24-hr collection of urine into a jar containing 20 ml of glacial acetic acid. The urine was kept refrigerated until delivery to the laboratory where an aliquot was obtained and frozen until the day of analysis. Patients receiving medications that interfered with the test were excluded from the study. The urinary excretion of kynurenine, 3-hydrokynurenine, kynurenic acid, xanthurenic acid, and acetylkynurenine was measured by the methods described by Price et al. (9).

Tryptophan pyrrolase activity was measured in 10- to 20-mg portions of Menghini liver biopsy specimens in 12 patients.

A liver specimen weighing about 0.5 g was obtained early in the operative procedure in the patients who underwent staging by laparotomy. Tryptophan pyrrolase was assayed in 15 of the specimens and kynureninase in 18.

Tryptophan pyrrolase activity was assayed by a modification of the micromethod of Altman and Greengard (1). The details and reasons for the modification are described elsewhere (7). In this method, the 100,000 x g supernatant from a 10- to 20-mg liver homogenate was incubated with tryptophan in a water bath at 37° with shaking in the presence of methemoglobin and a rat mitochondrial preparation. Tryptophan pyrrolase activity was calculated from the increase in absorbance at 360 nm after 30 min of incubation. The enzyme activity was expressed as μmoles of kynurenine formed per hr per 100 mg of soluble protein. Experiments performed on human liver specimens showed an increase in absorbance at 360 nm after 5 min of incubation, with a progressive increase up to 30 min of incubation. The levels of human hepatic tryptophan pyrrolase activity obtained by this method were distinctly higher than those reported by Altman and Greengard (1).

Hepatic kynureninase was assayed by either or both of 2 methods. In the 1st method, described by Dalgliesh et al. (4), kynureninase was determined by the disappearance of kynurenine from the reaction vessel, compared to a control incubation. In the 2nd method, described by de Castro et al. (5), the supernatant of a liver homogenate was incubated in the presence of L-kynurenine, and pyridoxal phosphate and anthranilic acid production were measured. In our earlier experience, the method of Dalgliesh et al. was used. Because kynureninase activity was frequently low in the livers of patients with various neoplasms, the method of de Castro et al. was adopted and used, in addition to the Dalgliesh method, in most of the assays in this series. The general agreement between the 2 methods will be shown in “Results.”

RESULTS

The urinary excretion of tryptophan metabolites following a 2-g dose of L-tryptophan was measured in 22 patients with Hodgkin’s disease prior to staging of the disease. On the basis of data obtained from the literature and the limited number of controls that we studied, the upper limits of “normal” for the excretion of tryptophan metabolites after a 2-g L-tryptophan load were set at 70 μmoles/24 hr for kynurenine, xanthurenic acid, and acetylkynurenine, and 100 μmoles/24 hr for 3-hydroxykynurenine and kynurenic acid. There was increased urinary excretion of kynurenine in 3 patients and of 3-hydroxykynurenine in 6 of the 11

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patients with Stage I or Stage II Hodgkin’s disease. One symptomatic (Group B) patient in this group tended to excrete larger quantities of tryptophan metabolites than the asymptomatic Group A patients (Chart 2). Increased excretion of kynurenine was found in 6, of 3-hydroxykynurenine in 10, of kynurenic acid in 4, of xanthurenic acid in 4, and of acetylkynurenine in 3 of the 11 patients with Stage III or IV disease. The increased excretion of these metabolites was often more marked in the patients with Stage III or IV disease, compared with the abnormalities found in the patients with more-limited Stage I or II Hodgkin’s disease.

Urinary excretion of these tryptophan metabolites, following a 2-g L-tryptophan load, was measured in 18 patients with lymphosarcoma; 2 had localized Stage I disease, while the rest had Stage III or IV disease. There was increased excretion of kynurenine in 5, of 3-hydroxykynurenine in 7, of kynurenic acid in 1, of xanthurenic acid in 1, and of acetylkynurenine in 5 of the 18 patients (Chart 3).

Tryptophan pyrrolase was assayed in needle biopsy specimens of the liver in 6 and in surgical biopsy specimens in 9 patients with Hodgkin’s disease. The range of enzyme activity was 1.4 to 5.6 μmoles kynurenine per 100 mg protein per hr in the liver specimens obtained from patients with Stage I or II Hodgkin’s disease and 2.3 to 7.1 μmoles kynurenine per 100 mg protein per hr in the liver of patients with Stage III or IV Hodgkin’s disease. There appeared to be no correlation between the stage of the disease and tryptophan pyrrolase activity. Similarly, no correlation could be found between the activity of this enzyme and the 24-hr urinary excretion of kynurenine (Chart 4). Tryptophan pyrrolase activity also was assayed in needle biopsy specimens in 8 patients and in surgical biopsy specimens in 7 patients with lymphosarcoma. The range of enzyme activity in the liver of patients with lymphosarcoma was 2 to 6.2 μmoles kynurenine per 100 mg protein per hr. Here again there appeared to be no correlation between the enzyme activity and the level of the urinary excretion of kynurenine or the excretion of the other tryptophan metabolites.

Kynureninase was assayed by the method reported by Dalgleish et al. in 11 surgical biopsy liver specimens obtained from patients with Hodgkin’s disease. The enzyme activity ranged from 0.12 to 0.65 μmole kynurenine consumed per 10 mg protein per hr (Chart 5). Kynureninase also was assayed in 8 of these specimens by the method of de Castro et al.; the range of activity was 0.062 to 0.316 μmole anthranilic acid formed per 10 mg protein per hr (Chart 5). There appeared to be a good correlation between the 2 methods used for the measurement of kynureninase. This enzyme activity tended to be lower in patients with the more widespread Stage III and IV Hodgkin’s disease. Moreover, the patients with low hepatic kynureninase activity tended to excrete larger amounts of kynurenine in the urine after a 2-g L-tryptophan load was administered (Chart 6).

Kynureninase also was assayed by both methods in 5 surgical biopsy liver specimens obtained from patients with lymphosarcoma (4 with histiocytic lymphosarcoma and 1 with lymphocytic lymphosarcoma), while an assay only by the method of de Castro et al. was performed on 2 additional liver specimens obtained from patients with histiocytic lymphosarcoma. The range of activity was roughly the same as that found in the liver of patients with Hodgkin’s disease (0.12 to 0.48 μmole kynurenine consumed per 10 mg protein per hr in Dalgleish’s method and 0.054 to 0.144 μmole anthranilic acid formed per 10 mg protein per hr in de Castro’s method). Once again kynureninase activity was highest (0.128 and 0.144 μmole anthranilic acid formed per 10 mg protein per hr) in the 2 patients with Stage I histiocytic lymphosarcoma.

**DISCUSSION**

This study confirmed the reported high incidence of increased urinary excretion of tryptophan metabolites in
patients with Hodgkin's disease or lymphomas. It also confirmed that these abnormalities were both more frequent and more pronounced in the widespread Stage III and IV, compared with the more limited Stage I and II Hodgkin's disease. A similar correlation between the extent of the disease and the increased excretion of tryptophan metabolites has been reported in patients with cancer of the breast (6) and of the bladder (7). Because of the limited number of patients with Stage I and II lymphosarcoma in this series, correlation between the stage of the disease and the extent of tryptophan abnormalities could not be ascertained.

The greater increase in the urinary excretion of tryptophan metabolites in advanced compared with limited breast cancer was interpreted by DeGeorge and Brown as evidence supporting the suggestion of Altman and Greengard that increased tryptophan pyrrolase activity is responsible for the abnormal excretion. This enzyme is inducible by glucocorticoids and it is conceivable that the stress of the disease state could result in increased activity of the enzyme in patients with more advanced cancer, resulting in an increase in the urinary excretion of tryptophan metabolites. However, contrary to observations in patients with rheumatoid arthritis and several other diseases (1), but similar to our observations in patients with bladder cancer (7), these tests indicated there was no correlation between tryptophan pyrrolase activity and urinary excretion of kynurenine in the patients with Hodgkin's disease or lymphosarcoma.

Several of the enzymes involved in the tryptophan-niacin pathway utilize pyridoxal phosphate as a cofactor. Chabner et al. (3) reported the frequent occurrence of low serum pyridoxal phosphate in patients with Hodgkin's disease, which they demonstrate was more frequent in the symptomatic patients and in the patients with the more extensive disease. In the present study we frequently observed a lower level of hepatic kynureninase activity in patients with Hodgkin's disease, in particular those with Stage III or IV disease, in comparison to levels reported for normal people (0.425 to 0.47 μM kynurenine consumed per 10 mg protein) (10). A similar trend was also found in the patients with lymphosarcoma. As in bladder cancer (7), the patients with low hepatic kynureninase tended to excrete increased quantities of kynurenine and some of the other tryptophan metabolites in the urine. This enzyme hypoactivity was not caused by vitamin B₆ deficiency, since an excess amount of pyridoxal phosphate was added to the assay system in vitro. This latter finding might explain the frequent persistence of some abnormalities of tryptophan-niacin metabolite excretion in patients with Hodgkin's disease who were treated with large doses of pyridoxine (3).

REFERENCES

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