Studies on Tryptophan Metabolism in Patients with Bladder Cancer

S. Gailani, G. Murphy, G. Kenny, A. Nussbaum, and P. Silvernail

Departments of Medicine [S.G., A.N., P.S.] and Urology [G.M., G.K.], Roswell Park Memorial Institute, Buffalo, New York 14203

SUMMARY

Measurement, after 2 g L-tryptophan load p. o., of urinary excretion of some of the metabolites of the tryptophan-niacin pathway was performed on 36 patients with bladder cancer. Assay of hepatic tryptophan pyrrolase activity was done on 21 patients, and assay of kynureninase was done on all patients. Of these patients, increased excretion of kynurenine was found in 12, 3-hydroxykynurenine was found in 25, kynurenic acid was found in 9, xanthurenic acid was found in 4, and acetylkynurenine was found in 10. There was no correlation between tryptophan pyrrolase activity and the level of the urinary excretion of tryptophan metabolites, while patients with low kynureninase activity tended to excrete increased quantities of these metabolites. Depressed kynureninase activity and increased excretion of tryptophan metabolites were more marked in the more advanced Stage D1 and D2 bladder cancer. Measurements of excretion of the tryptophan metabolites 6 to 12 months after eradication of the disease in 19 patients showed a statistically significant decrease in the excretion of kynurenine, while increased excretion of 3-hydroxykynurenine persisted in 9 of the patients. The excretion of the other metabolites was within normal range in all the patients postoperatively. Similar measurements on two other patients with known evidence of metastases showed an increase in the excretion of the various metabolites in the postoperative compared to the preoperative measurements.

INTRODUCTION

Most of the known industrial and experimental bladder carcinogens are aromatic amines or their metabolites (13). A search for endogenous bladder carcinogens led to extensive studies of urinary excretion of tryptophan metabolites in patients with bladder cancer (2, 3, 14). This work was given impetus by the finding of Dunning et al. (9) that almost 100% of Fischer line 344 rats developed bladder cancer when implanted with the diet containing 0.06% 2-acetylaminofluorene, whereas no bladder tumor developed when the diet was not supplemented with tryptophan.

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 1). 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 of these kynureninase, which catalyzes the conversion of kynurenine to anthranilic acid and of 3-hydroxykynurenine to 3-hydroxyanthranilic acid, would appear to play a key role.

Brown et al. (3) measured the urinary excretion of some of the metabolites of the tryptophan-niacin pathway after 2 g L-tryptophan load in 41 patients with bladder cancer; 20 of the patients excreted increased amounts of kynurenine, 3-hydroxykynurenine, acetylkynurenine, and kynurenic acid compared to normal controls. Subsequently, it was demonstrated that cholesterol pellets impregnated with 8-hydroxyquininalic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, xanthurenic acid, or the 8-methyl ether of xanthurenic acid produced a high incidence of bladder cancer when implanted into the bladder of mice (4). The finding that some of the abnormalities of tryptophan metabolite excretion in patients with bladder cancer might persist after eradication of the neoplasm (17) led to the speculation that abnormal tryptophan metabolite excretion in bladder cancer patients might represent a metabolic aberration in some of those patients that led to increased excretion of carcinogenic tryptophan metabolites with subsequent increased incidence of bladder cancer.

Altman and Greenberg (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 conceivably increased activity of this enzyme in some of the patients with bladder cancer is responsible for the elevated excretion of tryptophan metabolites. A possible alternative or additional factor might be a decrease in activity of some of the enzymes involved in this pathway, e.g., kynureninase, that would lead to increased accumulation of certain metabolites. In this investigation, an attempt was made to correlate hepatic tryptophan pyrrolase and kynureninase activity in patients with bladder cancer with the urinary excretion of some of the tryptophan metabolites after 2 g L-tryptophan load.
MATERIALS AND METHODS

Thirty-six patients with an established diagnosis of bladder cancer, who were admitted to Roswell Park Memorial Institute for investigation for possible total cystectomy and construction of ileal conduit, were subjected to the following investigative procedures after an informed consent was obtained.

A Menghini needle biopsy aspiration of the liver was obtained on the 1st 15 patients in this series. A small portion of the specimen was sent for histological examination and the remaining 10 to 20 mg were used for assay of tryptophan pyrrolase.

L-Tryptophan, 2 g, blended in ginger ale, was given at 9 a.m. or within 2 hr after the liver biopsy specimen was obtained (not later than 10:30 a.m.). This was followed by a 24-hr urine collection in a jar containing 20 ml of glacial acetic acid. Since all the patients had indwelling catheters as part of their preoperative management, completeness of the urinary collection was assured. The urine was kept refrigerated until delivery to the laboratory where an aliquot was obtained and frozen until the day of analysis. Patients with grossly bloody or infected urines and those receiving medications such as glucocorticoids, estrogens, or sulfonamides that might influence the excretion of tryptophan metabolites or interfere with the tests were excluded. The urinary excretion of kynurenine, 3-hydroxykynurenine, kynurenic acid, xanthurenic acid, and acetylkynurenine was measured by the methods described by Price et al. (15). The same urinary metabolites also were measured in 10 patients with cancer other than cancer of the bladder or lymphoma and in 5 normal men aged 20 to 30 years. Measurement of tryptophan metabolites after tryptophan loading was repeated on 21 of the bladder cancer patients 6 to 21 months after total cystectomy; 19 of the patients were clinically free of evidence of cancer, while 2 had definite evidence of metastases.

In those patients who had laparotomy, a liver specimen weighing about 0.5 g was obtained early in the operative procedure (usually within 20 min of the beginning of the operation) for assay of tryptophan pyrrolase and kynureninase.

Assay of tryptophan pyrrolase activity by the micro-method of Altman and Greengard (1) was first attempted. In this method the 100,000 x g supernatant of 10 to 20 mg liver homogenate was placed in a microcuvet with a 1-cm light path and was incubated at 37° with tryptophan in the presence of methemoglobin and rat liver mitochondrial preparation. Tryptophan pyrrolase activity was measured from the rate of increase of absorbance at 360 nm (absorption peak of kynurenine) in a Gilford spectrophotometer. The enzyme activity was expressed as μmoles of kynurenine formed per hr per 100 mg of soluble protein.

Assay of the 1st 3 human liver specimens by this method in our laboratory produced very little or no change in absorbance at 360 nm (absorption peak of kynurenine) in a Gilford spectrophotometer. The enzyme activity was expressed as μmoles of kynurenine formed per hr per 100 mg of soluble protein. Assay of the 1st 3 human liver specimens by this method in our laboratory produced very little or no change in absorbance at 360 nm (absorption peak of kynurenine) in a Gilford spectrophotometer.
absorbance at 360 nm. The supernatant of homogenates of human liver biopsies obtained during laparotomy from 4 patients with cancer other than cancer of the bladder was prepared as described by Altman and Greengard (1); 0.2-ml samples of the supernatant of the liver homogenates (obtained from 10 mg of liver) were placed in flasks and incubated with tryptophan, methemoglobin, and rat mitochondrial preparations in a water bath at 37° with shaking. Two such samples and a blank preparation containing these reagents but without the addition of liver were transferred to microcuvets prior to and after 5, 10, 15, and 30 min of incubation, and the spectrophotometric absorbance at 360 nm was measured. Increased absorbance became apparent after 5 min of incubation and progressed with time. Chart 2 indicates the linear increase in absorbance that was observed in 4 individual liver samples during the initial 30-min incubation period. No appreciable change of absorbance occurred in the blank preparations. Tryptophan pyrrolase activity calculated from the change in absorbance between 0 and 30 min in the 4 linear preparations was 3.2, 3.32, 3.4, and 4.9 μmoles kynurenine per 100 mg protein per hr. When enzyme activity was calculated from the difference in absorbance reading at 10 and 30 min of incubation, comparable results were obtained in 2 specimens, while in the other livers the results were higher by 10 and 15%. The results reported in these experiments for human liver tryptophan pyrrolase were distinctly higher than those reported by Altman and Greengard (1). The reason for this is unknown but may be related to the modification of the method. All subsequent calculations of tryptophan pyrrolase activity in this study were based on change in absorbance at 360 nm after 30 min of incubation.

Kynureninase was assayed in the earlier samples by the method of Dalgliesh et al. (6). Kynureninase was determined by the disappearance of kynurenine from the reaction vessel compared to a control incubation. Because kynureninase activity was frequently low in the livers of patients with bladder cancer, the method of de Castro et al. (7) was used for the assay of this enzyme in the last 16 samples. In this method the supernatant of a liver homogenate was incubated in the presence of excess L-kynurenine and pyridoxal phosphate and the anthranilic acid formed measured.

RESULTS

The urinary excretion of tryptophan metabolites, following a 2-g dose of L-tryptophan, was measured in 36 patients with bladder cancer prior to operation (Chart 3). On the basis of data obtained from the literature (3, 5) and the limited number of normal 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 at 100 μmoles/24 hr for 3-hydroxykynurenine and kynurenic acid. In the 36 patients with bladder cancer, there was increased excretion of kynurenine in 12, 3-hydroxykynurenine in 25, kynurenic acid in 9, xanthurenic acid in 4, and acetylkynurenine in 10. The pattern of excretion of the various metabolites in the 15 patients who received the tryptophan immediately after needle biopsy of the liver was similar to those who were not subjected to the procedure (Chart 4). Similar measurements were performed on 10 patients with cancer other than cancer of the bladder or lymphoma; there was increased excretion of kynurenine in 4, 3-hydroxykynurenine in 6, kynurenic acid in 1, xanthurenic acid in 2, and acetylkynurenine in 3 (Chart 3).

Increased excretion was seen in any stage of bladder cancer, but values in excess of 450 μmoles/24 hr (sum of the 5 tryptophan metabolites measured in the urine) were seen only in the more extensive Stage D1 and D2 disease (Chart 5).

Tryptophan pyrrolase activity was assayed in needle biopsy specimens of the liver of 15 patients with bladder cancer. In 10 of these patients, the enzyme was subsequently measured in a surgical biopsy specimen of the liver. In 6 other patients, the enzyme was assayed in samples of liver obtained by surgical biopsy only (Chart 6). The results

![Chart 2. Change in absorbance at 360 nm during assay of tryptophan pyrrolase in 4 human liver specimens.](chart2)

![Chart 3. Urinary excretion of kynurenine (K), 3-hydroxykynurenine (30H-K), kynurenic acid (KA), xanthurenic acid (XA), and acetylkynurenine (AcK) after 2 g L-tryptophan load in patients with bladder cancer (○) and cancer of other organs (●) and in normal controls (△).](chart3)
Tryptophan pyrroline was measured in 5 liver specimens obtained at laparotomy on patients with neoplasms other than cancer of the bladder or lymphoma. The range of enzyme activity in these liver specimens was 1.98 to 4.4 μmoles kynurenine per 100 mg protein per hr with a median of 2.94; this is similar to the range of enzyme activity found in the liver of patients with bladder cancer.

Kynureninase was assayed in 24 surgical biopsy specimens obtained from patients with bladder cancer by the method of Dalgliesh et al. (6). The values ranged from 0.118 to 0.56 μmole of kynurenine metabolized per 10 mg protein per hr. Kynureninase tended to be lower in patients with Stage D1 and D2 disease compared to those with less extensive disease (Chart 8). Twenty of these patients were given 2 g L-tryptophan, and the subsequent 24-hr urine specimens were assayed for tryptophan metabolites. Pa-
Tryptophan Metabolism in Bladder Cancer

Patients with low kynureninase activity tended to excrete larger amounts of kynurenine. A better correlation could be demonstrated when the sum of the amounts of kynurenine, 3-hydroxykynurenine, kynurenic acid, and acetylky
urenine excreted was compared with kynureninase activity (Chart 9). Patients with low kynureninase excreted larger amounts of these metabolites compared to those with high enzyme activity.

Kynureninase was assayed by the method of de Castro et al. (7) in the liver of 16 patients with bladder cancer. In these patients, the enzyme activity ranged from 0.062 to 1.24 μmoles anthranilic acid per 10 mg protein per hr (Chart 10). Patients with Stage D2 bladder cancer had the lowest activity. In 14 of these patients urinary tryptophan metabolites were measured, and again, patients with low kynureninase activity excreted increased amounts of tryptophan metabolites. Kynureninase measured by the latter method on 8 liver specimens obtained from patients with neoplasms other than cancer of the bladder or lymphoma ranged from 0.063 to 0.12 μmole per 10 mg protein per hr.

Kynureninase was measured by the methods of both Dalgliesh et al. and de Castro et al. in 4 liver samples (Table 1). There was good correlation between the 2 methods, although the absolute activities were different.

Five of the 36 patients with bladder cancer in this series had recurring bladder papillomas over a period of 4 to 9 years, and 6 patients had 3 to 8 local recurrences of cancer after excision over a period of 2 to 24 years. In 10 of these patients, the urinary excretion of tryptophan metabolites was within the normal range, a finding which fails to support a role for the increased excretion of these metabolites in the etiology of the recurring tumors.

Measurement of the urinary excretion of tryptophan metabolites was repeated in 19 patients 6 to 12 months after the eradication of their disease (Chart 11). All of these patients were clinically free of bladder cancer. The excretion of kynurenine was significantly lower in the postoperative period ($p < 0.01$). There was no statistical difference between the pre- and postoperative excretion of the other metabolites ($p > 0.05$), although the excretion of kynu-

![Chart 8. Hepatic kynureninase in patients with bladder cancer measured by the method of Dalgliesh et al.](image)

![Chart 9. Correlation of hepatic kynureninase (Dalgliesh et al.) and the sum of 24-hr urinary excretion of kynurenine, 3-hydroxykynurenine, kynurenic acid, and acetylky
urenine after 2 g L-tryptophan load in patients with bladder cancer.](image)

![Chart 10. Hepatic kynureninase measured by the method of de Castro et al. in patients with various stages of bladder cancer and in patients with cancer of other organs.](image)

Table 1

| Kynureninase measured by methods of Dalgliesh et al. and de Castro et al. in 4 liver specimens |
|---------------------------------|---------------------------------|
| Dalgliesh et al. | de Castro et al. |
| (μmole kynurenine utilized/10 mg protein/hr) | (μmole anthranilic acid/10 mg protein/hr) |
| 0.17 | 0.064 |
| 0.29 | 0.084 |
| 0.34 | 0.10 |
| 0.47 | 0.12 |
measurements were done on 2 other patients 8 and 10 months after total cystectomy at a time when there was definite evidence of recurrence of the neoplastic disease; in comparison to the preoperative period both patients showed an increase in the excretion of the various metabolites, including kynurenine.

**DISCUSSION**

The finding of frequent increased urinary excretion of tryptophan metabolites of the kynurenine pathway in patients with bladder cancer and the persistence of these abnormalities in some of the patients after eradication of the disease were taken as evidence that an aberration of tryptophan metabolism in some patients leads to an increased excretion of carcinogenic tryptophan metabolites and consequent high incidence of bladder cancer (13). The absence of similar abnormalities in 16 patients with industrial bladder cancer was used as additional evidence in support of the latter hypothesis (3). On the other hand, the demonstration by several workers of similar abnormalities in patients with bladder cancer who became clinically free of cancer. Similar pre- (Δ) and postoperative (○) measurements in patients who developed definite postoperative metastases.

In this study, the high incidence of increased excretion of tryptophan metabolites in patients with bladder cancer was similar to that reported by Brown et al. (3). Similar to the observations made in patients with Hodgkin's disease (5) and breast cancer (8), these abnormalities were more pronounced in patients with the more advanced D₁ and D₂ stages of the disease. Moreover, many of the patients who exhibited marked abnormalities died within 1 year.

The normal excretion of kynurenine, kynurenic acid, xanthurenic acid, and acetylkyurenine postoperatively in disease-free patients could support the argument that the abnormal tryptophan metabolism in bladder cancer patients is secondary to the disease process. However, the argument is weakened by the persistence of increased 3-hydroxykynurenine excretion in 9 of these patients. The low kynurenine and high 3-hydroxykynurenine excretion postoperatively might be the result of increased reabsorption of kynurenine compared to 3-hydroxykynurenine by the ileal conduit; in experiments with rat ileum the absorption of kynurenine was much more efficient than that of 3-hydroxykynurenine (16). The persistence of very high kynurenine excretion postoperatively in 2 other patients who had definite evidence of disease activity tends to exclude the reabsorption of kynurenine by the ileal conduit as the only cause for the restoration of kynurenine excretion to normal values. The persistence of increased 3-hydroxykynurenine might be the result of other clinical problems from which the patients suffered.

The greater increase in the excretion of tryptophan metabolites in advanced breast cancer compared to the more limited disease was interpreted by De George and Brown (8) as evidence supporting the suggestion of Altman and Greengard (1) that increased tryptophan pyrrolase activity is responsible for this effect. This enzyme is inducible by glucocorticoids, and it is conceivable that the stress of cancer could result in increased tryptophan pyrrolase activity and consequent increase in the excretion of tryptophan metabolites. The introduction of minor modifications to the micromethod of tryptophan pyrrolase assay resulted in a much higher level of this enzyme than that reported by Altman and Greengard (1). The range of tryptophan pyrrolase activity in the present series was 1.98 to 4.9 μmoles kynurenine per 100 mg protein per hr compared to 0.26 to 1.2 μmoles kynurenine per 100 mg protein per hr in the series of Altman and Greengard (1). Perhaps the higher activity in this series was the result of stress caused by cancer and hospitalization in our patients. However, the demonstration in our laboratory of a 3- to 4-fold increase in tryptophan pyrrolase activity in rat liver preparations assayed by our modification implies that the difference in the assay methods was responsible for the higher values for tryptophan pyrrolase in this series. Contrary to what has been reported in patients with rheumatoid arthritis and several other diseases (1), there was no correlation between tryptophan pyrrolase activity and the urinary kynurenine excretion in the cancer patients studied in this series. A similar lack of correlation was found between the activity of the enzyme and the excretion of the other metabolites. This lack of correlation could not be attributed to a possible difference of 15% in activity that might occur if the increase in absorbance was calculated after 30 min of incubation rather than by using the linear part of the increase in absorbance during the 1st 30 min to calculate this value.

The frequent observation of elevated kynurenine and 3-hydroxykynurenine in the urine of patients with bladder cancer led us to measure the level of hepatic kynureninase in patients with bladder cancer. Kynureninase catalyzes the conversion of kynurenine into anthranilic acid and 3-hydroxykynurenine into 3-hydroxyanthranilic acid (10).
We observed low activity of kynureninase in many patients with bladder cancer, particularly in the patients with the more advanced disease. This hypoactivity was not caused by vitamin B6 deficiency, since an excess amount of pyridoxal phosphate was added to the assay system in vitro. Patients with low kynureninase tend to excrete larger quantities of tryptophan metabolites in the urine. Thus, kynureninase may play a significant although not the sole role in the control of the level of various metabolites of the tryptophan-niacin pathway. Low activity of kynureninase also was observed in patients with other neoplasms.

REFERENCES

Studies on Tryptophan Metabolism in Patients with Bladder Cancer

S. Gailani, G. Murphy, G. Kenny, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/33/5/1071

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/33/5/1071.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.