Clinical Significance of Simultaneous Determination of Serum Tryptophan and Tyrosine in Patients With Lung Cancer

Ya-Ping Ren, Ai-Guo Tang,* Qian-Xuan Zhou, and Zhong-Yuan Xiang
Department of Clinical Laboratory, Second Xiangya Hospital, Central South University,
Changsha, P.R. China

Background: To explore the clinical significance of serum tyrosine (Tyr) and tryptophan (Trp) in patients with lung cancer, we used a simple and efficient method of high-performance liquid chromatography with fluorescence detection (HPLC-FD) that simultaneously measured serum Trp and Tyr contents. **Methods:** The concentrations of Tyr and Trp were measured simultaneously by HPLC-FD in the sera of 80 patients with lung cancer and 120 healthy controls. **Results:** Trp concentrations were significantly lower in patients with lung cancer than in healthy controls (39.26 ± 5.44 vs. 49.93 ± 5.43 $\mu\text{mol/l}$, respectively; $P < 0.01$), whereas in Tyr concentrations there were no differences with healthy controls (65.38 ± 7.94 vs.

66.40 ± 8.55 $\mu\text{mol/l}$, respectively; $P > 0.05$). In addition, patients in the adenocarcinoma group had significantly lower Trp and Tyr concentrations than those in squamous cell carcinoma group. There was no difference between the early stage and advanced stage of lung cancer. **Conclusions:** Determination of serum Trp and Tyr concentrations can be employed to assist the diagnosis of the histotypes of lung cancer and tumor stage. Tyr and Trp as indexes on the lung cancer diagnostic sensitivity, specificity were 54.9, 62.9% and 82.4, 92.1%, Trp is an important and special index for lung cancer diagnosis of which the specificity of diagnosis of lung cancer is more than 92%. J. Clin. Lab. Anal. 25:246–250, 2011. © 2011 Wiley-Liss, Inc.

Key words: lung cancer; serum; tryptophan; tyrosine

INTRODUCTION

Lung cancer is one of the most common cancers in the world. It is a leading cause of cancer death in human. As early as the 19th century, the people had taken into account the diet or nutritional status related to the body pathogenesis of tumor (1). L-tryptophan (Trp) is an essential amino acid and L-tyrosine (Tyr) is an unessential amino acid. Both are abundant protein components and act as precursors for several biologically important compounds (2–4). The degradation of Trp in vivo proceeds through two major biochemical pathways: one is the biosynthesis of the neurotransmitter serotonin and the other is the transformation to kynurenine (kynurenine pathway, KP) (5–7). At least two enzymes, tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase (IDO), are involved in the KP (6,8,9). Studies have shown that the concentrations of Tyr and Trp in serum are closely related with numerous diseases in neuropsychiatric system (10–13), immune system (14), tumors (15–17), liver diseases (18,19), and critically ill patients (20). In patients with tumor, malnutrition, and excessive consumption (cancer cells

rapid proliferation, accelerated protein synthesis in tumor tissue) can cause Tyr and Trp metabolic disorders. Disease occurrence, such as viral infection, autoimmune disorders, and tumor, may also induce a number of amino acid metabolism enzymes, including IDO, to accelerate degradation of amino acids, including Trp and Tyr. Through the analysis of the concentrations of Tyr and Trp in lung cancer patient serum, we study the metabolism of Trp and Tyr in lung cancer cases, and explore the clinical significance of serum Tyr and Trp with the auxiliary diagnosis and pathological staging classification of lung cancer.

Grant sponsor: Science and Technological Department of Hunan Province; Grant number: 05SK3026; Grant sponsor: The Public Health Department of Hunan Province; Grant number: C2005019.

*Correspondence to: Ai-Guo Tang, Department of Clinical Laboratory, Second Xiangya Hospital, Central South University, 139 Renming Zhong Road, Changsha 410011, P.R. China.

E-mail: aiguoatang@yahoo.com.cn

Received 31 October 2010; Accepted 30 March 2011

DOI 10.1002/jcla.20467

Published online in Wiley Online Library (wileyonlinelibrary.com).

MATERIALS AND METHODS

Subjects

Eighty patients with lung cancer (44 males and 36 females, mean ages 55.7 years) were enrolled in this study who recruited from the Department of chest surgery of Second Xiangya Hospital of Central South University. No patients had autoimmune diseases, viral hepatitis, chronic renal failure, or human immunodeficiency virus infection. All patients were staged according to the 1997 International Union against Cancer criteria (21), and were divided into two subgroups: early and advanced lung cancers. Early and advanced lung cancers were defined as stages I, II and stages III, IV, respectively. Histological typing was classified according to the World Health Organization criteria (22). One hundred and twenty healthy control subjects (62 males and 58 females, mean ages 52.5 years) who came from for health check-up in the Second Xiangya Hospital of Central South University (free from any physical illness and normal laboratory findings in blood chemistry, chest X-ray, and ECG). The sample collection and use for this study have been approved by the ethic committees of Second Xiangya Hospital of Central South University.

Laboratory Examinations

At the time of blood sampling, the patients had not received any treatment, including operation, chemotherapy, or radiation therapy. Blood samples were collected in procoagulant tube in the morning and centrifuged at 3,000g for 5 min to obtain serum samples. All serum samples were stored at -20°C until analysis. Routine laboratory examination, such as blood cell counts and biochemical analysis, was performed.

Measurements of Trp and Tyr Concentrations in Serum

Measurement of Trp and Tyr concentrations in serum from lung cancer group and control group was performed by HPLC and fluorimetric detection. Serum samples were deproteinized by mixing with an equal volume of 5% (v/v) perchloric acid, followed by vortex-mixing for 10 min and centrifuged at 10,000g for another 10 min at 4°C . Ultimately, 20 μl of the supernatant was injected into the HPLC system for analysis. The stock solutions of Tyr (5,500 $\mu\text{mol/l}$) and Trp (4,900 $\mu\text{mol/l}$) were prepared by 2.5% (v/v) perchloric acid, respectively. Standard working solutions of mixed Trp and Tyr were made by further dilution of each standard stock solution to 2.5% (v/v) perchloric acid, and the working solutions were prepared fresh the same day as they were used. Referred to the foreign literature method (23,24), we improved and established a new HPLC-FD according to our laboratory

conditions. The chromatographic analysis was performed using a Megres C18 column (250 mm $\times$ 4.6 mm i.d., 5 μm ; Hanbon Sci. Tech. Co., Jiangsu, China). The chromatographic conditions were as follows: 10% (v/v) acetonitrile as the mobile phase, a flow rate of 1.2 ml/min, column temperature at 25°C . The fluorescence was recorded at the optimal wavelength for Tyr ($\lambda_{\text{ex}} = 228 \text{ nm}$, $\lambda_{\text{em}} = 306 \text{ nm}$) from 0 to 5 min, and the optimal wavelength for Trp ($\lambda_{\text{ex}} = 285 \text{ nm}$, $\lambda_{\text{em}} = 353 \text{ nm}$) after 5 min. The linearity ranges were from 0.275 to 275 $\mu\text{mol/l}$ (Tyr) and from 0.49 to 196 $\mu\text{mol/l}$ (Trp). The limit of detection was found to be 0.004 $\mu\text{mol/l}$ for Tyr and 0.005 $\mu\text{mol/l}$ for Trp, respectively. The recovery was 90.5–108.8% and 88.8–97.2% for Tyr and Trp, respectively. The precision results showed that the relative standard deviation of intraday and interday was $<5\%$, respectively. Phenylalanine, 5-hydroxytryptamine, kynurenic acid, kynurenine, or creatinine did not interfere with the determination of Tyr and Trp.

Statistical Analysis

Eighty hospitalized patients with lung cancer and 120 healthy controls were analyzed using the developed method. The data were presented as mean $\pm$ SD and analyzed using SPSS11.0 statistical software. *P* values less than 0.05 were considered as statistically significant.

RESULTS

Clinical Characteristics

The clinical characteristics of the 80 patients with lung cancer are summarized in Table 1, 47 patients were classified as early lung cancer and 33 patients were classified as advanced lung cancer. Seventy-six patients had nonsmall cell carcinoma (40 adenocarcinomas (ADC), 36 squamous cell carcinomas (SCC)), whereas 4 patients had small cell carcinoma.

Serum Concentrations of Trp and Tyr

Concentrations of Trp and Tyr are presented in Table 2. Trp concentrations in patients were significantly lower than in controls ($P < 0.01$), whereas Tyr concentrations were only slightly lower in patients. No significant difference was found between the concentration of Trp and Tyr in the ages of patients or differentiations of lung cancer.

Tyr and Trp as Indexes on the Evaluation of Diagnosis of Lung Cancer

Tyr and Trp as indexes on the lung cancer diagnostic sensitivity, specificity, positive predictive value, negative predictive value were 54.9, 62.9, 33.3, 80.5% and 82.4, 92.1, 71.2, 93.8%, Trp which as an index on the

TABLE 1. Clinical Characteristic of Patients With Lung Cancer

Variables	Number of cases
Number of patients	80
Ages	
≤ 55	33
> 55	47
Gender	
Male	44
Female	36
Histotype	
ADC	40
SCC	36
SCLC	4
Staging	
Early lung cancer	47
Stage I	29
Stage II	18
Advanced lung cancer	33
Stage I	29
Stage II	4
Differentiation	
Poor	18
Moderate	47
Well	15

TABLE 2. Concentrations of Tyr and Trp in Serum of Control Group and Lung Cancer Group

Group	C (Tyr) (μmol/l)	C (Trp) (μmol/l)
Control	66.40 ± 8.55	49.93 ± 5.43
Lung cancer	65.38 ± 7.94	39.26 ± 5.44 ^a

^aThere is a significant difference between the lung cancer group and the control group, $P < 0.01$.

specificity of diagnosis of lung cancer is more than 92% (shown in Table 3).

Correlation of Serum Trp and Tyr With Disease Histological Types

Patients suffering from ADC tended to have lower Tyr and Trp (63.17 ± 6.17 and 38.25 ± 5.17 μmol/l, respectively) as compared with patients suffering from SCC (66.90 ± 6.13 and 41.32 ± 3.51 μmol/l, respectively) ($P < 0.05$), (Fig. 1). Patients with ADC had both lower Tyr and Trp concentrations ($P < 0.01$) than healthy controls (66.40 ± 8.55 and 49.93 ± 5.43 μmol/l, respectively). Patients with SCC had lower Trp concentrations than healthy controls, yet their Tyr levels were comparable.

Association of Serum Trp and Tyr With Disease Stages

Comparing patients with early and advanced lung cancer and the healthy controls, significant decreases in

TABLE 3. Tyr and Trp as Indexes on the Evaluation of Diagnosis of Lung Cancer (%)

	Sensitivity	Specificity	Predictive value (PV)	Negative predictive value (NPV)
Tyr	54.9	62.9	33.3	80.5
Trp	82.4	92.1	71.2	93.8

Fig. 1. Comparison of concentration of Tyr and Trp between ADC, SCC, and healthy controls. ADC, adenocarcinoma; SCC, squamous cell carcinoma.

serum concentration of Trp ($P < 0.01$) were found in patients with early and advanced lung cancers than healthy controls. Patients with advanced lung cancer had lower Tyr concentrations than healthy controls, but there is no significant difference ($P > 0.05$). Staging dependency of Tyr or Trp concentrations was insufficient. There was no difference between early and advanced lung cancers. Among each factor of TNM classification, the serum concentrations of Trp were significantly lower in T4 than in T1 (Table 4), without a significant difference in the concentrations of Tyr. Regarding N and M factors, the serum concentrations of Tyr and Trp had no significant difference.

DISCUSSION

This study simultaneously measured the serum concentrations of Tyr and Trp using HPLC-FD, and investigated the correlation between the concentrations of Tyr and Trp and the clinical parameters of lung cancer. It was found that patients with lung cancer had significantly lower Trp concentrations than healthy controls. Accelerated Trp catabolism has been described in several malignant diseases, such as solid tumors and hematological neoplasias (17,25). A decrease in Trp

TABLE 4. Comparison Between Serum Concentrations of Tyr and Trp and Disease Stages

Staging	C(Tyr)($\mu\text{mol/l}$)	C(Trp)($\mu\text{mol/l}$)
Early lung cancer ($n = 47$)	66.17 ± 5.42	39.63 ± 6.38
Advanced lung cancer ($n = 33$)	64.26 ± 7.46	38.25 ± 4.69
<i>T</i>		
T1 ($n = 19$)	66.48 ± 7.2	41.04 ± 3.42
T2 ($n = 26$)	65.39 ± 8.35	39.99 ± 5.82
T3 ($n = 20$)	65.01 ± 6.18	38.29 ± 6.81
T4 ($n = 15$)	64.46 ± 7.39	$37.03 \pm 4.37^*$
<i>N</i>		
N0 ($n = 37$)	65.16 ± 8.38	39.62 ± 6.60
N1 ($n = 21$)	65.28 ± 7.67	38.52 ± 5.11
N2 ($n = 18$)	66.01 ± 7.96	39.36 ± 5.67
N3 ($n = 4$)	65.10 ± 6.98	39.34 ± 4.85
<i>M</i>		
M0 ($n = 76$)	65.43 ± 8.89	39.28 ± 6.21
M1 ($n = 4$)	64.43 ± 6.86	38.88 ± 4.63

* $P < 0.01$, T1 vs. T4.

suggests an enhanced cytokine-induced degradation of Trp and an activated IDO mechanism. Furthermore, T-cell proliferation and immune response could be inhibited by an increased IDO activity in lung cancer patients. The activation of cellular immune system was reported to be associated with colorectal cancer (26), gynecological cancer (17), and breast cancer (27). Meanwhile, malnutrition in patients with lung cancer and excessive consumption (cancer cells rapid proliferation, accelerated protein synthesis in tumor tissue) can cause disordered Tyr and Trp metabolism. Referring to the previous study: there were many literatures about Trp degradation in tumor, but no report about lung cancer. Engin AB recently reported about Trp metabolic changes in patients with lung cancer (28), and observed the enhanced degradation of Trp. Similar to Engin AB's research, we also found that Trp concentrations were significantly lower in patient with lung cancer than in controls.

Little is known about the correlation of Tyr and Trp concentrations and clinicopathological parameters in lung cancer. We found that patients with advanced lung cancer had no significant high concentrations than those with early lung cancer, indicating that staging dependency of Tyr or Trp concentrations was insufficient. Regarding TNM classifications, we found no significant decrease in patients with different classification. We also examined a correlation between Tyr and Trp concentration and disease histological types. The results showed that ADC had significant lower Tyr and Trp concentrations than those with SCC, which may be related to the way of proliferation and metastasis of cancer.

Lung cancer is one of the most common cancers in the world. It is a leading cause of cancer death in human. There is no generally accepted screening test for lung cancer. The diagnosis is usually made on the basis of

clinical criteria and nonspecific laboratory findings. The lack of a rapid and accurate screening method may delay the optimal time for therapy of the disease. In this study, we observed that the concentrations of Tyr and Trp in patients with lung cancer are under the normal levels of healthy group. Determination of serum Trp and Tyr concentrations can be employed to assist the diagnosis of the histotypes of lung cancer and tumor stage. Larger clinical trials will be necessary to find out the potential prognostic expressiveness of Tyr and Trp metabolism in patients suffering from lung cancer.

CONCLUSIONS

We have applied this method on analyzing the concentrations of Tyr and Trp in serum of lung cancer patients and found decreased levels of Trp and Tyr by the lung cancer, which provides a potential diagnosis index for clinicians. Determination of serum Trp concentrations can be employed to assist the diagnosis of the histotypes of lung cancer and tumor stage. Although Tyr did not have the same influence on the lung cancer as Trp, Tyr concentrations can be employed to assist the diagnosis of ADC and SCC. Tyr and Trp as indexes on the lung cancer diagnostic sensitivity, specificity were 54.9, 62.9% and 82.4, 92.1%, Trp is an important and special index for lung cancer diagnosis which the specificity of diagnosis of lung cancer is more than 92%.

REFERENCES

1. Luo WC. Modern Respiratory. Beijing: People's Medical Publishing House, 1997. p 789–825.
2. Vaarmann A, Kask A, Mäeorg U. Novel and sensitive high-performance liquid chromatographic method based on electrochemical coulometric array detection for simultaneous determination of catecholamines, kynurenine and indole derivatives of tryptophan. *J Chromatogr B* 2002;769:145–153.
3. Amirkhani A, Heldin E, Markides KE, Bergquist J. Quantitation of tryptophan, kynurenine and kynurenic acid in human plasma by capillary liquid chromatography-electrospray ionization tandem mass spectrometry. *J Chromatogr B* 2002;780:381–387.
4. Myint AM, Kim YK, Verkerk R, et al. Kynurenine pathway in major depression: Evidence of impaired neuroprotection. *J Affect Disord* 2007;98:143–151.
5. Pertovaara M, Raitala A, Uusitalo H, et al. Mechanisms dependent on tryptophan catabolism regulate immune responses in primary Sjögren's syndrome. *Clin Exp Immunol* 2005;142: 155–161.
6. Maneglier B, Rogez-Kreuz C, Cordonnier P, et al. Simultaneous measurement of kynurenine and tryptophan in human plasma and supernatants of cultured human cells by HPLC with coulometric detection. *Clin Chem* 2004;50:2166–2168.
7. de Jong WH, Smit R, Bakker SJ, et al. Plasma tryptophan, kynurenine and 3-hydroxykynurenine measurement using automated on-line solid-phase extraction HPLC-tandem mass spectrometry. *J Chromatogr B* 2009;877:603–609.

8. Schröcksnadel K, Wirleitner B, Winkler C, Fuchs D. Monitoring tryptophan metabolism in chronic immune activation. *Clin Chim Acta* 2006;364:82–90.
9. Fukushima T, Mitsunashi S, Tomiya M, et al. Determination of kynurenic acid in human serum and its correlation with the concentration of certain amino acids. *Clin Chim Acta* 2007;377:174–178.
10. Pawlak D, Pawlak K, Malyszko J, et al. Accumulation of toxic products degradation of kynurenine in hemodialyzed patients. *Int Urol Nephrol* 2001;33:399–404.
11. Hartai Z, Klivenyi P, Janaky T, et al. Kynurenine metabolism in plasma and in red blood cells in Parkinson's disease. *J Neurol Sci* 2005;239:31–35.
12. Hartai Z, Juhász A, Rimanóczy A, et al. Decreased serum and red blood cell kynurenic acid levels in Alzheimer's disease. *Neurochem Int* 2007;50:308–313.
13. Ehrlich S, Franke L, Schneider N, et al. Aromatic amino acids in weight-recovered females with anorexia nervosa. *Int J Eat Disord* 2009;42:166–172.
14. Widner B, Sepp N, Kowald E, et al. Enhanced tryptophan degradation in systemic lupus erythematosus. *Immunobiology* 2000;201:621–630.
15. Travers MT, Gow IF, Barber MC, et al. Indoleamine 2,3-dioxygenase activity and L-tryptophan transport in human breast cancer cells. *Biochim Biophys Acta* 2004;1661:106–112.
16. Feder-Mengus C, Wyler S, Hudolin T, et al. High expression of indoleamine 2,3-dioxygenase gene in prostate cancer. *Eur J Cancer* 2008;44:2266–2275.
17. Schroeksnadel K, Winkler C, Fuith LC, Fuchs D. Tryptophan degradation in patients with gynecological cancer correlates with immune activation. *Cancer Lett* 2005;223:323–329.
18. Ma L, Xu B, Wang W, et al. Analysis of tryptophan catabolism in HBV patients by HPLC with programmed wavelength ultraviolet detection. *Clin Chim Acta* 2009;405:94–96.
19. Dejong CH, van de Poll MC, Soeters PB, et al. Aromatic amino acid metabolism during liver failure. *J Nutr* 2007;137:1579S–1585S.
20. Pandharipande PP, Morandi A, Adams JR, et al. Plasma tryptophan and tyrosine levels are independent risk factors for delirium in critically ill patients. *Intensive Care Med* 2009;35:1886–1892.
21. Watanabe Y. TNM classification for lung cancer. *Ann Thorac Cardiovasc Surg* 2003;9:343–350.
22. Gibbs AR, Thunnissen FB. Histological typing of lung and pleural tumours: Third edition. *J Clin Pathol* 2001;54:498–499.
23. Khan RI, Amin MR, Mohammed N, Onodera R. Quantitative determination of aromatic amino acids and related compounds in rumen fluid by high performance liquid chromatography. *J Chromatogr B* 1998;710:17–25.
24. Sánchez-Machado DI, Chavira-Willys B, López-Cervantes J. High-performance liquid chromatography with fluorescence detection for quantitation of tryptophan and tyrosine in a shrimp waste protein concentrate. *J Chromatogr B* 2008;863:88–93.
25. Karanikas V, Zamanakou M, Kerenidi T, et al. Indoleamine 2,3-dioxygenase (IDO) expression in lung cancer. *Cancer Biol Ther* 2007;6:1258–1262.
26. Brandacher G, Perathoner A, Ladurner R, et al. Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: Effect on tumor-infiltrating T cells. *Clin Cancer Res* 2006;12:1144–1151.
27. Travers MT, Gow IF, Barber MC, et al. Indoleamine 2,3-dioxygenase activity and L-tryptophan transport in human breast cancer cells. *Biochim Biophys Acta* 2004;1661:106–112.
28. Engin AB, Ozkan Y, Fuchs D, et al. Increased tryptophan degradation in patients with bronchus carcinoma. *Eur J Cancer Care* 2010;19:803–808.