

Role of tryptophan in the pathogenesis of hepatic encephalopathy

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Abstract

Objective: To monitor serum levels of total and free tryptophan in various hepatic dysregulations.

Method: The study was conducted on 36 adult patients. All were suffering from acute or chronic liver diseases such as hepatitis (n= 12), cirrhosis (n= 12) and Hepatic Encephalopathy (HE) (n=12). Patients of all age groups and both genders were included. Serum levels of Tryptophan (TRP) were measured by HPLC-EC. Albumin and bilirubin were analyzed by kit method (Merck).

Results: The serum levels of total TRP ($p<0.01$) was found in all patients. Free TRP significantly ($p<0.01$) increased only in HE. Patients with hepatitis and cirrhosis did not show significant change in serum free TRP. The Albumin levels significantly decreased in hepatitis, cirrhosis and in HE ($p<0.01$). All patients exhibited hypoalbuminaemia ($p<0.01$).

Conclusions: The results suggest that an increased ratio of free to bound TRP enhances its availability to the brain, which in turn increases 5-HT synthesis thus precipitating encephalopathy (JPMA 58:66;2008).

Introduction

Tryptophan an essential amino acid is the precursor of 5-HT. It is the only amino acid that occurs in protein bound form.¹ TRP hydroxylase, the rate limiting enzyme of 5-HT biosynthesis exists unsaturated with its substrate therefore, the availability of TRP to the brain affects the synthesis of 5-HT.² TRP and other large neutral amino acids (LNAAs) are transported into the brain via common carrier system located on the blood brain barrier.^{3,4} About 90% of the circulating TRP exists in protein bound form.⁵ Fractions of the TRP that exists in unbound form and the concentrations of LNAAs other than TRP have been shown to have important impact on the availability of TRP to the brain. 5-HT is implicated in consciousness and sleep.⁶ An increase in the availability of TRP to the brain could enhance synthesis of 5-HT that may lead to the elicitation of sleep.⁶⁻⁸ Increases in brain 5-HT and 5-hydroxy indoleacetic acid (5-HIAA), the principle metabolite of 5-HT have been also observed in animal models of HE due to fulminant hepatic failure.⁹⁻¹¹ The present study is designed to investigate the role of plasma TRP in the elicitation of encephalopathy associated in the liver dysfunctions.

Patients and Methods

This study was carried out on 36 adult patients admitted to the hospital over an 8 months (May-Dec 2006) period. There were 18 males and 18 females. The age range was between 17 and 64; with a mean age of 41.8 ± 11.9 years. Patients were divided into three groups based on etiology of their hepatic disease process. Group 1 consisted of 12 patients with chronic liver disease due to hepatitis

viral type B and C. Group II consisted of 12 patients with cirrhosis of the liver, with no evidence of encephalopathy referred to as stable cirrhosis. They had sustained superimposed insults such as GIT bleeding, infection etc. Group III consisted of 12 patients in stage III or IV hepatic encephalopathy according to the classification of Trey's et al.¹² Diagnosis of the disease based on history and clinical picture in all cases of each group. In addition to their being encephalopathy, the patients met two of the following four inclusion criteria.

- I. Serum bilirubin >2.5 mg/100ml.
- II. SGOT > 80 μ /100ml.
- III. Prothrombin time 2.5 sec prolonged.
- IV. Serum albumin >3.0 mg/100ml.

Blood ammonia concentration was elevated in most patients of all groups. Control consisted of 12 subjects with no evidence of liver disease. Sample from normal volunteers were also collected in the same hospital. Body weights, age and sex etc were matched. Since heights of the subjects were not matched so instead of BMI, body weight was taken. All experiments were performed according to an approved local ethical committee protocol. The levels of TRP (total and free) and albumin were estimated. Albumin and bilirubin were estimated by albumin Merck kit and bilirubin Merck kit. Serum total and free TRP were determined by HPLC - EC. A5II Shim-Pack ODS separation columns of 4.0 mm internal diameter and 150 mm length were used. Separation was achieved by a mobile phase containing methanol (14%), Octyl sodium sulphate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer

of pH 2.9 at an operating pressure of 2000 -3000 psi on Shimadzu HPLC-EC 6A detector at an operating potential of 1.0 volts for TRP. Ultrafiltrate were prepared for the estimation of serum free TRP. For this purpose specifically prepared cell fitted with dialysis membrane cone were used. Serums in the cone were centrifuged to get ultrafiltrate. Samples for analysis were stored at - 70°C.

Data were analyzed by one-way ANOVA. The results were considered statistically significant when $p < 0.05$.

Results

Table 1 shows age, sex, bodyweight and bilirubin in patients suffering from hepatitis, cirrhosis and HE. Values for control volunteers are also given in the same Table.

Table 2 shows serum levels of total TRP, free TRP and free/total TRP ratio and albumin levels in hepatitis (n=12), cirrhosis (n=12), and HE (n=12) respectively. Values for control volunteers are also given in the same table. Data analyzed by one - way ANOVA showed a significant decrease ($p < 0.01$) in total TRP in hepatitis, cirrhosis and HE. Free TRP levels significantly ($p < 0.01$) increased in HE and not in Cirrhosis and Hepatitis. Ratio of Free /total TRP was significantly higher ($p < 0.01$) in HE but this increase was not significant in Hepatitis and Cirrhosis. Serum albumin levels were significantly ($p < 0.01$) smaller

Table 1. Age, sex and body weight and bilirubin of patients suffering from acute or chronic liver disease and of normal controls.

Subject	n=48	Male n=26	Female n=22	Age (years)	Body Wt. Mean (Kg)	Bilirubin (mg %)
Controls	12	8	4	41.8±9.4	62.5±4.5	0.52±0.19
Hepatitis	12	8	4	44.4±11.3	59.1±3.2	18.01±7.4
Cirrhosis	12	6	6	47.4±6.4	61.5±6.6	0.73±3.4
HE	12	4	8	44.4±12.4	57.3±5.5	10.2±5.8

Table 2. Serum levels of total, free trp, free/total trp ratio and albumin in hepatic dysregulations.

Parameters	Control	Hepatitis	Cirrhosis	HE	1 - way ANOVA df = 44
Total TRP(mg/ml)	10.16±3.8	**4.78±1.22	**4.48±1.2	**5.6±0.9	F = 20.393 $p < 0.01$
Free TRP(mg/ml)	1.57±0.42	1.94±0.87	1.3±0.5	**2.18±0.69	F = 5.145 $p < 0.01$
Free/Total TRP	0.27±0.2	0.392±0.98	0.34±0.2	*0.39±0.1	F = 10.057 $p < 0.05$
Albumin(g%)	5.13±0.089	**3.02±0.57	**3.32±0.7	**2.89±0.3	F = 54.38 $p < 0.01$

Values are means ± SD.

Significant difference by Newman-Keuls test

* $P < 0.05$ ** $P < 0.01$ from controls following one-way ANOVA

in patients with Hepatitis, Cirrhosis and HE.

Discussion

Hepatic encephalopathy is a serious neuropsychiatric complication that occurs in both acute and chronic liver failure. Clinical features include confusion, which is often associated with a flapping tremor, drowsiness, stupor and coma.¹³ Alterations in the metabolism of brain monoamines neurotransmitter 5-HT, its precursor TRP in experimental animals and human liver failure.⁹⁻¹¹ Brain 5-HT concentration have been shown to be extremely sensitive to brain TRP levels which in turn are regulated by the transport of TRP from the plasma via blood brain barrier.^{3,4} In some early studies it has been reported that serum levels of total TRP were higher in patients with various hepatic dysregulation.^{14,15} Animal research also showed an increase in circulating levels of TRP in thioacetamide (TAA) induced fulminant hepatic failure.^{11,16,17} A significant decrease in plasma albumin concentration together with low plasma TRP levels has been reported in patients with HE.¹⁵ Increase in free TRP concentration with normal serum total TRP levels in hepatic cirrhosis have also been reported in patients with acute hepatitis, cirrhosis and HE.^{1,18} We report a decrease in serum total TRP concentration in patients with acute hepatic cirrhosis and HE. In view of studies showing greater than normal brain 5-HT metabolism in postmortem samples of patients with HE⁶ and TAA induced HE in animals.^{16,19} it was expected that plasma levels of TRP will increase. Indeed plasma levels of tryptophan were higher in TAA induced HE in animals.¹⁶ A decrease in plasma TRP levels as observed in patients with hepatitis, cirrhosis and HE suggests that plasma total TRP may not contribute in the enhanced brain 5HT metabolism in HE, hepatitis and cirrhosis. Free TRP monitored in this study was significantly higher in HE whereas, in hepatitis and cirrhosis TRP levels were insignificant.

The availability of TRP in the circulation is dependent on its hepatic degradation^{16,20} via enzyme TRP pyrrolase, which is the major catabolic enzyme of TRP. It has been reported¹⁶ that the activity of TRP pyrrolase is increased in HE induced by TAA due to fulminant hepatic failure in rats.²¹ A decrease in serum total TRP levels as observed in the present study is therefore explainable in terms of an increase in TRP pyrrolase activity in the liver.

Brain TRP and 5-HT concentration are regulated by the relative concentration of plasma total TRP and other large neutral amino acid (LNAAs) sharing a common transport system with TRP.²² An Increase in concentration of one of these amino acid can decrease the transport of another amino acid.²³ Patients with chronic liver diseases

had elevated levels of aromatic amino acid whereas branched chain amino acids were consistently depressed.¹³ The increase in serum free TRP concentration in the present study might be involved in increased transport of this amino acid via LNAs carrier system in patients with HE. It has also been reported that increase of plasma TRP in hepatic dysregulation could increase 5-HT synthesis in the brain.^{3,4} TRP is the only amino acid which binds to albumin in the plasma.²⁴ Increase in free TRP concentration in cirrhosis is often explained in terms of impaired binding of this amino acid with the albumin.²⁵ In chronic liver diseases especially in liver cirrhosis and in HE the liver function is reduced and serum albumin is low.²⁴ Increase in free TRP concentrations as observed particularly in HE may be attributed to the decrease serum albumin levels in the present study would increase its transport in various tissue including the brain and the liver.^{15,24}

In conclusion the present study shows that an increase in free TRP levels in circulation particularly in HE increases the availability of TRP to the brain for the synthesis of serotonin and an increase in serotonin function could possibly be involved in the precipitation of unconsciousness and comatose condition in patients with hepatic dysregulations.

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