Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **42**, 93-99 (1999)

Study of Liver Function Tests Using Various Stable Isotope Labelled Compounds in Liver Cirrhosis

Akio Sakamoto, Yoshihiro Nabeya[1)], Kaichi Isono[1)]

Narutou General Hospital
167 Narutou Narutou-machi, Sanbu-gun, Chiba 289-1326, Japan
[1)] Department of Surgery, Chiba University School of Medicine

Summary

3 different stable isotope compounds which are metabolized in various sites of the liver were used to examine assessment methods which permit a precise expression of liver functions. The following 3 tests were performed in 17 cirrhotic patients and 16 healthy subjects as controls; ^{13}C -tyrosine breath test, ^{15}N-ammonium chloride oral loading test, ^{13}C-aminopyrine breath test. The ratio of cirrhotic patients to controls was 89.0 % (N.S.), 39.5 % ($p<0.001$), 32.9 % ($p<0.001$) in ^{13}C-tyrosine breath test, ^{15}N-ammonium chloride oral loading test, ^{13}C-aminopyrine breath test respectively. In cirrhotic patients the microsome function was most severely damaged, which is expressed by the ^{13}C-aminopyrine breath test. The result of the Student's t test of ^{13}C-aminopyrine breath test was -9.722 , which was higher than that of the ^{15}N-ammonium chloride oral loading test, -5.033. Accordingly, it was recognized that the ^{13}C-aminopyrine breath test most reflected a degree of disorder as a liver function test and also showed the highest reliability.

Key words

^{13}C-tyrosine breath test, ^{15}N-ammonium chloride oral loading test, ^{13}C-aminopyrine breath test, liver cirrhosis

CCC 0362–4803/99/010093–07$17.50

Received 3 November 1997
Revised 10 August 1998
Accepted 12 August 1998

Introduction

It is very important to understand the state of hepatic functions of cirrhotic patients since it was reported that liver functions will strongly reflect their vital prognosis (1). However, it is hard to measure liver function quantitatively by routine laboratory biochemical blood tests. To assess which testing method can examine liver function state most accurately, we chose these 3 important hepatic functions such as mitochondrion function, urea cycle function and smooth endoplasmic reticular function, i.e. microsome function to study accuracy of the liver function tests in degrees of hepatic disorder by determining these functions in cirrhosis patients in a safe manner with the use of stable isotope compounds which are free from radioactive pollution.

Subjects and methods

HUMAN SUBJECTS

The study was conducted in seventeen cirrhosis patients and sixteen healthy subjects as controls without history of liver disease and abnormalities in general blood biochemical tests. Cirrhotic patients were diagnosed as cirrhosis pathohistologically (Table 1).

group	age	serum albumin (g/dl)	GOT (IU/L)	GPT (IU/L)	total bilirubin (mg/dl)	ICG-R15 (%)
control	52±7	4.3±0.2	23±7	17±13	0.7±0.2	not done
cirrhosis	56±4	3.5±0.5[a]	86±36[a]	73±52[b]	1.2±0.5	30.2±12.5

Table 1. Standard tests of liver function in patients with cirrhosis and controls. (Mean ±S.D.) Stastistical difference from controls: [a] $p<0.001$, [b] $p<0.05$

Investigation was made by separately administering 3 stable isotope compounds metabolized at different sites of the liver (L-tyrosine-1-^{13}C, ammonium chloride-^{15}N, aminopyrine-N,N-dimethyl-$^{13}C_2$) to an individual subject on the different day for each compound. A 1-week interval was set between administration of L-tyrosine-1-^{13}C and aminopyrine-N,N-dimethyl-$^{13}C_2$. The stable isotope compounds were prepared at the pharmacy of the hospital as follows; L-tyrosine-1-^{13}C (99 excess atom %) (ICON, Summit, USA) and L-tyrosine (Ajinomoto, Tokyo, Japan) were dissolved in distilled

water which contained 0.9% NaCl to yield a concentration of 10 mg/ml after ethyl-esterified. Aminopyrine-N,N-dimethyl-$^{13}C_2$ (99 excess atom %) (ICON, Summit, USA) was also dissolved in distilled water which contained 0.9% NaCl to yield a concentration of 20mg/ml. All solutions were sterilized at 115℃ for 30 minutes with an autoclave after filtration with a 0.22μm filter. The subjects refrained from eating from the previous night and the examination was conducted in the fasting state in the early morning.

In ^{13}C-tyrosine breath test both 1mg.kg of L-tyrosine-1-^{13}C and 1mg/kg of L-tyrosine were administered through the cubital vein by bolus injection. Breath samples were collected in a poly vinyl fluoride film bag (GI Science, Tokyo, Japan) every 30 minutes for 3 hours after injection and 100 ml samples were withdrawn and injected into a vacutainer and ^{13}C excess atom % in breath was determined using a dual inlet isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany).

In the ^{15}N-ammonium chloride oral loading test 10 mg/kg of ^{15}N-ammonium chloride (99 excess atom %) (Shoko Co., Tokyo, Japan) was administered orally together with 300 ml distilled water and urine was collected in a period of 5 hours in a collection bag which contained 3 ml of 0.1N-HCl. Two ml of the 5 hours urine sample was separated into urea and ammonia fractions using a microdiffusion method (2). One ml sample of each fraction was nitrogen-gasified after oxidation-reduction in the nitrometer (Yanagimoto, Kyoto, Japan) and ^{15}N excess atom % in the nitrogen gas was measured with an isotope ratio mass spectrometer (Nichiden-Aneruba, Tokyo, Japan) to determine the ratio of ^{15}N excess atom % in the urea and ammonia fractions.

In the ^{13}C-aminopyrine breath test 2 mg/kg of aminopyrine-N,N-dimethyl-$^{13}C_2$ was injected as a volus via cubital vein and breath collections were performed before and at 15, 30, 45, 60, 90 and 120 minutes after injection. Breath samples were collected and analyzed in a manner similar to that employed for the ^{13}C-tyrosine breath test.

The tests chosen will assess metabolic function , but will not detect other manifestations of hepatic injury, e.g. lipid accumulation or biliary excretion.

CALCULATIONS

The rates of labeled CO_2 excretion at each time point were calculated in units of per cent of administered dose excreted as breath $^{13}CO_2$ per hour according the formulas reported by Watkins et al. (3). A CO_2 production of 300 m moles per meter^2 body surface area per hour reported by Shreeve et al. (4) was used as an excretion rate of carbonic acid gas to determine the recovery rate of 13-carbon metabolized and excreted in breath. The cumulative excretion was calculated by summing the trapezoidal areas under $^{13}CO_2$ excretion time-course curve. All results were expressed as the mean ± S.D..

A statistically significant differences were investigated between the controls and the patients with cirrhosis in Student's t test. As a result, a significant difference was recognized with $p<0.05$.

Results

The ^{13}C-tyrosine breath test showed that the time-course of $^{13}CO_2$ excretion values were slightly-lower in the cirrhosis group than in the control group but there was no recognized significant difference between them at any point during 3 hours (Figure 1). The average of 3-hour cumulative excretion was 10.0 ± 2.5 %, 8.9 ± 2.4 % in the controls, cirrhotic patients respectively. The average value of the patients decreased to 89.0 % of that of the controls but there was no significant difference between them, so that the ^{13}C-tyrosine breath test is not useful clinically (Figure 2).

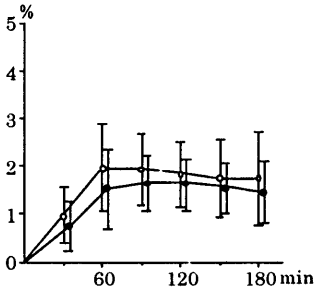

Figure 1. Time-course of $^{13}CO_2$ excretion in breath 3 hours after ^{13}C-tyrosine administration in (○) normal controls, and (●) patients with cirrhosis. (Mean ± S.D.)

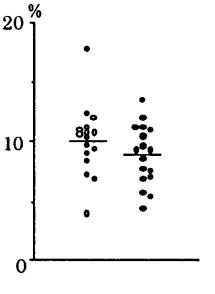

Figure 2. Cumulative $^{13}CO_2$ excretion in breath 3 hours after ^{13}C-tyrosine administration in (○) normal controls, and (●) patients with cirrhosis. The transverse lines represent the mean.

Figure-3 shows the ratio of ^{15}N excess atom % in urea fraction and ammonium fraction during urine accumulation 5 hours after oral administration of ^{15}N-ammonium chloride. The average of the ratio of ^{15}N excess atom % in urea fraction and ammonium fraction was significantly lower in the patient group with 0.47 ± 0.34 than that of the control group with 1.19 ± 0.47 ($p<0.001$). The average value of the cirrhosis group dropped to as low as 39.5 % of that of the control group. However, the ratios of 7 in 17 cirrhotic patients were overlapped with those of control subjects, this test could not separate between cirrhotic and control subjects clearly.

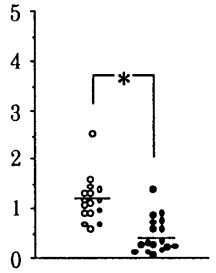

Figure 3. Ratio of ^{15}N excess atom % in urea fraction and ammonia fraction in urine in (○) normal controls, and (●) patients with cirrhosis. The transverse lines represent mean. Statistical difference from controls: * $p<0.001$; t values= −5.033

The time-course of $^{13}CO_2$ excretion in breath was significantly lower in the cirrhosis group than the control group in the ^{13}C-aminopyrine breath test. The value of the cirrhosis group shifted within the significantly-lower levels than that of the control group at any point after administration of ^{13}C-aminopyrine (Figure 4). As shown in Figure-5, the 2-hour cumulative excretion in breath was remarkably lower in the cirrhosis group than the control group (2.8 ± 1.7 % versus 8.5 ± 1.8 %, $p<0.001$) and the average of the

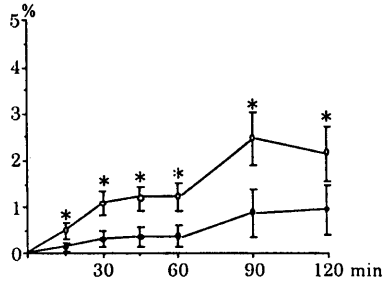

Figure 4. Time-course of $^{13}CO_2$ excretion in breath 2 hours after ^{13}C-aminopyrine administration in (○) normal controls, and (●) patients with cirrhosis. (Mean ±S.D.) Statistical difference from controls: * $p<0.001$

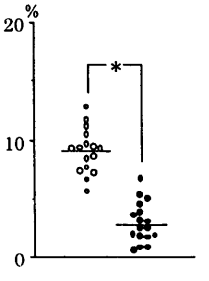

Figure 5. Cumulative $^{13}CO_2$ excretion in breath 2 hours after ^{13}C-aminopyrine administration in (○) normal controls, and (●) patients with cirrhosis. The transverse lines represent the mean. Statisticaldifference from controls: * $p<0.001$; t value= − 9.722

patient group dropped as low as 32.9 % of the control group. This test exhibited the lowest value among the three testing methods, and there was only one overlap between the results for ^{13}C-aminopyrine in cirrhotic versus normal subjects.

Discussion

It is well-known that tyrosine is mainly metabolized in liver and serum tyrosine level is increased in liver cirrhosis. However, it has been reported that no significant difference was observed in tyrosine metabolism in cirrhotic patients (5, 6). This relevant study also suggested that the mitochondrion function should be maintained in cirrhotic patients because there was no significant difference in tyrosine oxidation at any point during the examination with enough loads, 2mg/kg-BW of the total administered amount of tyrosine, and the 3-hour cumulative excretion rate of $^{13}CO_2$ in breath was 89.0 % of the normal liver group without any large difference.

The ratio of ^{15}N-urea excess atom % in urea fraction and ammonia fraction in 5-hour accumulated urine decreased to 39.5% of the controls and a remarkable drop in the urea cycle was also recognized in the ^{15}N-ammonium chloride oral loading test. In spite of the report (7) proposing that the gastric empty time is delayed in some of cirrhotic patients, therefore, false positive can be brought, it is considered that if any delay of gastric emptying, the results will not be affected because ^{15}N-ammonium chloride remained in the accumulated urine 5 hours after oral administration. Accordingly, the ^{15}N-ammonium chloride loading test turned out to be an accurate parameter for degree of hepatic disorder. This test can not be conducted in all patients since there are a large number of cirrhotic patients with hyperammonemia.

Breath tests with radioisotope- or stable isotope-labeled aminopyrine are widely used and significant correlations of the ^{13}C-aminopyrine breath test with BSP test, ICG test and serum albumin were reported (8, 9). As a strength, the aminopyrine breath test is free from influences of ascites (10). Although aminopyrine demethylation declines with aging (11) and delayed gastric emptying appears in some cases with cirrhosis (7), such problems

are unlikely to have happened in this study because there were no differences in age between the controls and the patients, and intravenous administration of aminopyrine. It is considered that a high reliability can be given to the ^{13}C-aminopyrine breath test, being judged by the higher t value in the ^{13}C-aminopyrine breath test in the study of a significant difference from the ^{15}N-ammonium chloride oral loading test (t value: -5.033, -9.722 respectively) and less overlap versus controls.

Conclusion

It was concluded that the ^{13}C-aminopyrine breath test via vein is a clinically-excellent, useful liver function test with accuracy and high reliability.

References

1.Villeneuve J.P., Infante-Rivard C., Ampelas M., Huet P.M., and Marleau D. — Hepatology 6: 928 (1986)

2.Jung K., Hirschberg K., Faust H., and Matkowitz R. — Eur J Nucl Med 11: 62 (1985)

3.Watkins J.B., Klein P.D., Scholler D.A., Park R. and Perman J.A. — Gastroenterology 82: 911 (1982)

4.Shreeve W.W., Shoop J.D., Ott D.G. and McInteer B.B. — Gastroenterology 78: 98 (1976)

5.Morgan M.Y., Hawley K.E. and Stambuk D. — Aliment Pharmacol Thera 4: 183 (1990)

6.O'Keefe S.J.D., Abraham R., El-Zayadi A., Marshall W. and Williams R. — Gastroenterology 81: 1017 (1981)

7.Schoeller D.A., Baker A.L. and Monroe P. — Hepatology 2: 455 (1982)

8.Hepner G.W. and Vesell E.S. — Ann Intern Med 83: 632 (1975)

9.Villeneuve J.P., Arsene D., and Huet P.M. — Clin Invest Med 6: 5 (1983)

10.Mion F., Queneau P.E. and Rousseau M. — Hepatogastroenterology 42: 931 (1995)

11.Pirotte J. and El Allaf D. — Digestion 28 : 21 (1983)

