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^{13}C-Breath Tests in hepatology (cytosolic liver function)

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Abstract. – **^{13}C-phenylalanine (PheBT) and ^{13}C-galactose breath tests (GBT) explore non invasively the hepatic functional mass by measuring two enzymatic activities localized into the cytosol of liver cells: the phenylalanine hydroxylase (which converts phenylalanine into tyrosine) and the galactose kinase (which catalyzes the ATP-dependent phosphorylation of galactose to galactose 1-phosphate). Both BTs are safe and accurate in predicting the severity of liver cirrhosis showing a good correlation with the Child-Pugh score. PheBT is also used in predicting postoperative complications and monitoring liver regeneration in patients undergoing partial hepatectomy. GBT has been also used to assess liver fibrosis in patients with chronic hepatitis C. PheBT and GBT could be used in the diagnosis of two inborn errors of metabolism, phenylketonuria and galactosemia, respectively. Both BTs are not affected by enzymatic induction due to drugs which may interfere with the results of the classic "microsomial" BTs (such as the aminopyrine or caffeine BTs).**

Key Words:

Phenylalanine, Galactose, Breath test, Cytosol.

Introduction

The liver is the principal site of aromatic amino acid metabolism, and the plasma concentrations of these amino acids (phenylalanine and tyrosine) are largely dependent upon liver function[1].

Phenylalanine Breath Test

Phenylalanine participates in a number of metabolic pathways, the major one being irreversible hydroxylation to tyrosine by phenylalanine hydroxylase in the liver. Three other pathways of phenylalanine metabolism, normally of minor quantitative importance, are transamination to phenylpyruvic acid, decarboxylation to form β-phenylethylamine, and acetylation of the amino group. In phenylalanine metabolism, the hydroxylation of phenylalanine to tyrosine is rate-limiting, so that the ^{13}C-PheBT may allow estimation of the *in vivo* rate of phenylalanine hydroxylation. Tyrosine may be hydroxylated to form dihydroxyphenylalanine (DOPA), iodinated to form triiodothyronine or decarboxylated to form tyramine. The major metabolic pathway for tyrosine, however, is transamination to *p*-hydroxyphenylpyruvic acid. Most *p*-hydroxyphenylpyruvic acid is converted to homogentisic acid and, at this step, the label of L-[1-^{13}C]phenylalanine and L-[1-^{13}C]tyrosine is released as $^{13}CO_2$. In tyrosine metabolism, the transamination of tyrosine to *p*-hydroxyphenylpyruvic acid is rate-limiting, and about 99% of the daily degradation of tyrosine normally flows through *p*-hydroxyphenylpyruvic acid to homogentisic acid. Therefore, ^{13}C-TyrBT may allow estimation of the *in vivo* rate of tyrosine transamination. Both phenylalanine hydroxylation and tyrosine transamination take place within the liver and are significantly suppressed in patients with liver dysfunction. Indeed, several studies showed that liver disease is commonly associated with abnormal elevations of the plasma concentrations of phenylalanine and tyrosine and abnormally low breath excretion of $^{13}CO_2$ after oral administration of either L-[1-^{13}C]phenylalanine or L-[1-^{13}C]tyrosine[2].

The clinical usefulness of ^{13}C-PheBT (or ^{13}C-TyrBT) has been explored in several studies (Table I). Burke et al. first reported that ^{13}C-PheBT results are correlated with scores of the Child Pugh classification, which is widely accepted as a predictor of the sever-

Table I. Risk factors for chronic viral hepatitis.

Application	Species	Ref
^{13}C-Phenylalanine-BT		
Prediction of the severity of liver cirrhosis (correlation with Child score)	Homo s	3
Prediction of postoperative complications in patients undergoing hepatectomy	Homo s	4
Monitoring liver function after partial hepatectomy (regeneration marker)	Rat	5
Identification of patients with phenylketonuria	Homo s	9
^{13}C-Galactose-BT		
Prediction of the severity of liver cirrhosis (correlation with Child score)	Homo s	13
Assessment of liver fibrosis in patients with chronic hepatitis C	Homo s	14
Monitoring liver function after acute ethanol administration (?)	Rat	16
Identification of patients with galactosemia	Homo s	17

ity of liver disease[3]. The ^{13}C-PheBT is sensitive enough not only to assess the hepatocyte functional reserve in patients with liver disease[4] but also to predict postoperative complications in patients undergoing hepatectomy[5]. Moreover, the test is able to monitor liver function after partial hepatectomy in rats and might be proposed as a dynamic marker of hepatic regeneration[6]. Unfortunately, the ^{13}C-PheBT is unable to distinguish between patients with Child-Pugh A cirrhosis and patients with chronic hepatitis[7-8] thus not allowing a non invasive measure of the degree of hepatic fibrosis. Finally, an interesting application of ^{13}C-PheBT could be the identification of patients without liver disease affected by phenylketonuria (PKU). In a very old study, the PheBT was able to discriminate between normals and PKU heterozygotes and between normals and classic phenylketonurics with a classification error rate comparable to the gold standard test (i.e.: the fasting serum L-phenylalanine over L-tyrosine ratio)[9].

The advantages of the PheBT (or TyrBT) over the "classic" liver BTs exploring the hepatocyte microsomial function (such as aminopyrine, phenacetin, caffeine, and methacetin BTs) are remarkable. First, the two BTs are not affected by enzymatic induction due to drugs (cimetidine, erythromycin, rifampicin, phenobarbital, etc) which may interfere with the results of the classic "microsomial BTs". Secondly, no side effects have been ever reported with the use of phenylalanine or tyrosine (in U.S. the aminopyrine BT is not currently approved by the FDA due to the potential risk of fatal agranulocytosis[10]). Some concern exists, however, in severe liver failure where phenylalanine hydroxylase is deficient and phenylalanine is converted into tyramine and octopamine by alternative synthetic pathways. These compounds are false neurotransmitters and might cause or worsen hepatic encephalopathy. However, so far no mental deterioration in cirrhotic patients has been ever reported after oral administration of L-[1-^{13}C]phenylalanine, at least at the usual single dose (100 mg) used in breath testing.

Galactose Breath Test

Galactose is mainly metabolized in humans in the liver, the rate limiting step being the galactose kinase, an enzyme located in the cytosol of hepatocytes[11]. The sinusoidal membrane of the hepatocyte has a high extraction capacity for this sugar: when galactose is given at low dose, its hepatic metabolism depends mainly on the hepatic blood flow, and thus large doses are needed to saturate the metabolic pathway and give relevant information on liver function mass[12].

The clinical usefulness of ^{13}C-GBT has been explored in a few studies (Table I). Saadeh et al. showed that the ^{13}C-GBT is able to distinguish between normal and cirrhotic patients and also between Child-Pugh class A cirrhotics and Child-Pugh class B and C cirrhotics. Therefore it could be useful as a predictor of survival in patients with chronic liver disease, although it adds little to the Child-Pugh score[13]. Mion et al. reported that the ^{13}C-GBT results are decreased in patients with HCV-related chronic hepatitis and are inversely related to the severity of liver fibrosis[14]. In this sense, it could be used as a prog-

nostic factor in the follow-up of patients with chronic hepatitis C.

Before applying this test to patients with liver diseases, theoretical limitations of the test must be kept in mind. For example, ethanol can interfere with the galactose metabolism, through inhibition of the epimerase transforming galactose-1-P into glucose[15]. In rats, Mion et al. have shown that acute ethanol administration does significantly decrease $^{13}CO_2$ production from ^{13}C-galactose[16]. Diabetes may also interfere with ^{13}C-GBT, especially when hyperglycemia is present: in this case, ^{13}C-glucose produced from ^{13}C-galactose is diluted in the enlarged glucose pool, leading to a decreased $^{13}CO_2$ production. When using ^{13}C-GBT in diabetic patients, it is thus of importance to take into account the serum level of glucose when ^{13}C-GBT is performed to obtain relevant information on liver function.

Finally, an interesting application of ^{13}C-GBT is the identification of patients with galactosemia, a rare inborn error of the metabolism of galactose due to deficiency or absence of galactose-1-phosphate uridyltransferase (GALT). Berry et al. showed that ^{13}C-GBT distinguishes among several GALT genotypes and may evaluate the extent of impaired galactose metabolism in patients with different GALT mutations[17]. It may be useful in establishing whether a patient with GALT mutation is capable of manifesting disease or not[17].

The advantages of ^{13}C-GBT over the "classic" liver BTs exploring the hepatocyte microsomial function are the same as reported for the ^{13}C-PheBT. However, because of the high cost of labeled substrate, the ^{13}C-GBT is very expensive, thus limiting its applicability to the clinical daily practice.

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