Evaluation of the 13C-aminopyrine breath test using nondispersive infrared spectrometry

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Abstract

Objective : **the aim of the study was to assess the value of the 13C Aminopyrine Breath Test (ABT) when performed using the NonDispersive InfraRed Spectrometry (NDIRS), which is a simple and cheap alternative to the mass spectrometry.**

Methods : **The results obtained by using the NDIRS method for performing the ABT were compared to the results obtained by a reference method, the 14C Aminopyrine Breath Test. For this purpose, in 32 patients admitted for various liver problems, an ABT was performed by using the 2 methods simultaneously. The repeatability of the results obtained at 120 minutes by the NIDRS method as compared to the 14C test was assessed by the method of Bland and Altman.**

Results : **The mean of difference between the results obtained by both methods at 120 minutes was 0.06 ± 0.46. The coefficient of repeatability between the two tests was 0.92 for a confidence interval of 95%. A good correlation (r = 0.93) was found between all individual results obtained in breath samples at different times of collection (30, 60, 90, 120 minutes), and between the results obtained at 120 minutes for both 13C and 14C tests (r = 0.94).**

Conclusion : **The 13C ABT performed using NDIRS is a valid alternative to the 14C technique in routine clinical practice.** (Acta gastroenterol. belg., **2000**, 63, **328-330**).

Introduction

The Aminopyrine Breath Test (ABT) is a simple, reliable and non-invasive radionuclide method for estimating the functional capacity of the hepatic microsomal oxidative system (1,2).This test is a strong predictor of short- and long-term survival in patients with liver cirrhosis (3-6).

The test can be performed using aminopyrine labelled with 14C, but the long half-life of this radionuclide constitutes a main obstacle for a widespread clinical use of this test, particularly in children.

To avoid these problems, aminopyrine labelled with 13C can also be used. The 13C is a stable non-radioactive isotope, but, until recently, the quantitation of 13C required a very sensitive and equally expensive technology, the isotope ratio mass spectrometry. The high costs of these analyzers and the need for skilled staff were clear limitations to use this technology in routine clinical practice.

The recently developed nondispersive infrared spectrometry (NIDRS) on the other hand is a simple and a cheap technique, and does not require skilled technicians. It is therefore well suited for clinical use. Initial studies in urea breath test suggest that the clinical value of NDIRS for the measurement of 13C in breath is equal to that of isotope ratio mass spectrometry (7). To our knowledge, there are no data available in the literature concerning the use of NDIRS for 13C ABT.

The aim of the present study was to assess the clinical value of the 13C ABT as measured with a new, commercially available NDIRS analyzer, with 14C test as reference method.

Material and methods

Following a clinical protocol approved by our ethical comittees, all patients hospitalized in the Liver and Gastroenterology Unit from February 13, 1999, to March 22, 1999, and fulfilling the inclusion criteria, were included in the study. The inclusion criteria were : admission in the Unit for liver biopsy in a context of liver tests disturbances (abnormal transaminases values persisting for more than 3 months, suspected alcoholic hepatitis, and suspected liver metastases on ultrasonography), age less than 70 years, ability to undergo an ABT.

Using these criteria, 32 patients were selected out of 35. Three patients were excluded from the study (1 case of severe encephalopathy, one case of acute bleeding, one acute liver failure). Mean age of the patients was 48.3 (range : 31-70) and male/female ratio was 2.2. Liver biopsies showed : normal liver (3 cases), alcoholic steatosis (6), alcoholic hepatitis (4), liver cirrhosis (16), liver metastases (1), drug induced cholestasis (2).

The ABT was performed during the hospital stay. Both tests with 14C-aminopyrine and 13C-aminopyrine were performed simultaneously. Fasting patients were given an oral dose of 2 microCi 14C-aminopyrine mixed with 75 mg aminopyrine labelled with 13C. Patients were instructed to stay in bed prior to and during the ABT in order to keep metabolic CO_2 production at a basal level. Breath samples were collected at time 0, 30, 60, 90, and 120 minutes.

Concerning the 14C method, samples were collected in a counting vial containing 2 ml of 1 M hyamine hydroxide in methanol and 2 ml ethanol. Thymolphtalein was used as indicator. 14C radioactivity was measured in a liquid scintillation counter. Results were

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expressed as a percentage of the ingested dose excreted per hour. This value is obtained by multiplying the 14C-aminopyrine excreted as breath $14CO_2$ by the endogenous CO_2 output (9 mmol/kg per hour) (8). In our center and using this method, values in normal subjects at 120 minutes are > 4%.

Concerning the 13C technique, samples were collected in bags, directly connected to the spectrometer for 13C activity measurement . Using this NDIRS analyzer (IRIS analysator, Wagner Analyzen Technik, Worpswede, Germany), the 13C data have been evaluated by the IRIS software procedure, which standardizes CO_2 production for the individual patient by using body heigh/ body weight, and calculates metabolism rate (% 13C/ hour) and cumulated metabolisation (% 13C cumulated dose) to obtain standardized comparable data from patient to patient.

The difference in values obtained in the different histologic groups was assessed by the Wilcoxon test.

The repeatability of the test by the NDIRS method as compared to the 14C method was assessed by the method of Bland and Altman (9).

Results

Mean values of 14C ABT at 120 minutes were 3.93%/h ± 2.47 (min : 0.3, max : 8.6), whereas those of 13C ABT were 3.94%/h ± 2.58 (min : 0.2, max : 8.1).

The values obtained at 120 minutes by both methods were highly correlated (r = 0.94). A good correlation was also found between the individual values obtained at the different times of collection (30, 60, 90, and 120 minutes) (r = 0.93) (Fig.1).

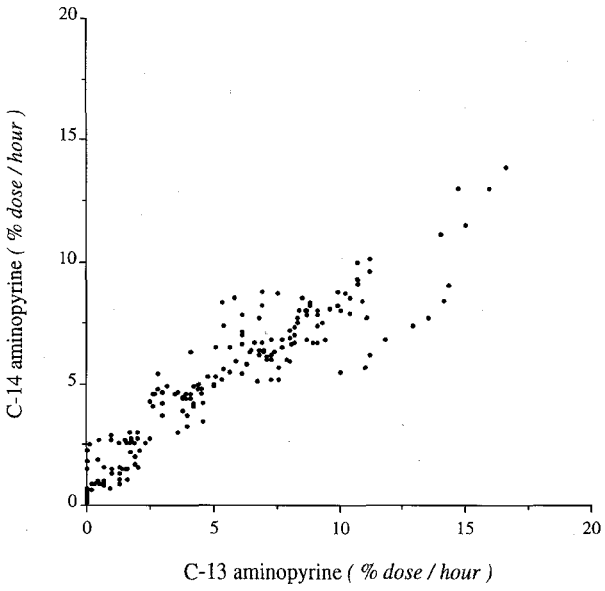

Fig. 1. — Correlation between the individual values of each patient at each collection time for both 13C and 14C tests (r = 0.93). Results are expressed in %/h excreted of ingested dosis.

The mean values of 13C ABT and 14C ABT at 120 minutes in cirrhosis were respectively 2.1%/h ± 1.8 and 2.0%/h ± 1.6. This difference was not significant.

The mean values of 13C ABT and 14C ABT in steatosis were respectively 6.9%/h ± 1.0 and 7.1%/h ± 1.1.

The difference between cirrhosis and steatosis was significant for both tests ($p < 0.001$, Wilcoxon test).

The plot of the mean difference and the mean values obtained by both tests is presented in Fig. 2. The mean of difference between the results obtained by 13C ABT and 14C ABT at 120 minutes was 0.06 ± 0.46. The coefficient of repeatability was 0.92 for a confidence interval of 95%.

Discussion

The ABT has been shown to be a reliable test for estimating the functional capacity of the liver microsomal oxidative system (1,2). This non-invasive procedure provides objective numerical data and constitutes a strong predictor of short- and long-term survival in patients with liver cirrhosis (3-6). The method using 14C is however hampered by the long half-life time of this radionuclide. The problem of radiation constitutes the main obstacle for a widespread clinical use of this test, especially in children. The 13C labelled aminopyrine is an attractive alternative. The tracer is a stable non-radioactive isotope, but, unfortunately, the quantitation of 13C requires the isotope ratio mass spectrometry. The high cost of this device and the need for skilled staff were clear limitations to this technology. The newly developed nondispersive infrared spectrometry (NDIRS) on the other hand is a simple technique which does not require skilled technicians. It may therefore prove to be an interesting alternative in routine clinical practice. Although new mass spectrometers with reduced price and easier manipulation recently have become available, the NDIRS system remains cheaper (about 2/3 of the price of a mass spectrometer, ± 40.000 Euro versus ± 56.000 Euro). The NDIRS system requires a larger volume of the breath samples as compared to the mass spectrometer that requires only 10 ml. This could be a limitation for the technique in some patients such as young children or for transportation of the breath samples.

For the urea breath test, the NDIRS has been studied and considered to be a valuable alternative to the mass spectrometry (7, 10, 11). Our study was conducted because no published data are available concerning the application of this new technology to liver function assessment by breath test. One difference with the 14C test is the fact that for 14C, only a tracer dosis of aminopyrine is needed, while a pharmacological dosis is needed (about 75 mg) for the 13C test. While no haematological toxicity has been reported since the first application of the 13C test by using the mass spectrometry in 1978 (12) , this potential complication has to remain in mind (13).

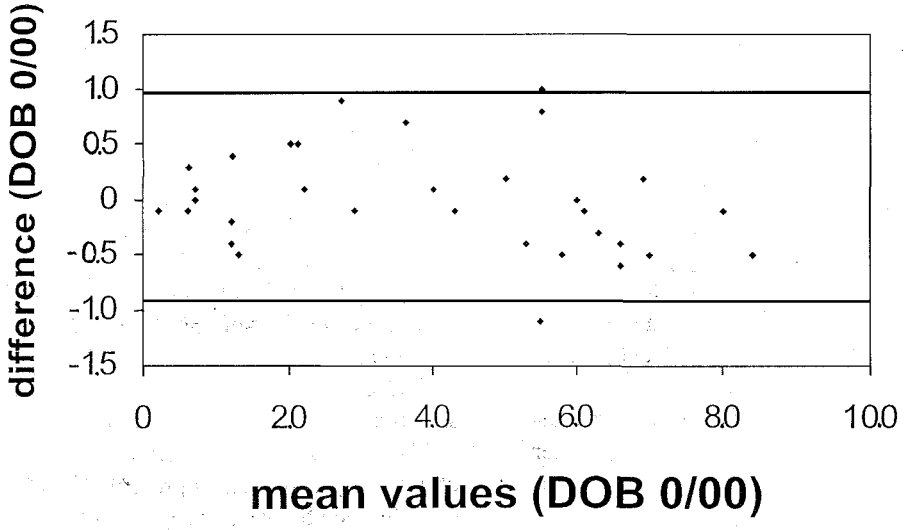

Fig. 2. — Bland and Altman plot of the results of 2 successive ABT with 13C and 14C. No bias was found between the two methods. SD of the difference was 0.46. The coefficient of repeatability between two successive tests was 0.92 for an interval of confidence of 95%.

Our results indicate the clinical value of the NDIRS technique for liver function assessment. There are different manners to expose the results of the ABT (14). We choosed the 120 minute collection according to several investigators (3, 5, 8). As we obtained a nice correlation between the individual values at the different times of collection, one can also expect the different manners to express the results to be similar.

We conclude that the close correlation between the results of 13C and 14C methods suggests that the NDIRS may be used for clinical purpose instead of both 14C, and 13C mass spectrometry techniques. However, the stability of the instrument and of the measurements have to be confirmed in larger, well-documented studies.

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