

Clinical application of ^{13}C -Hiolein breath test in assessing pancreatic exocrine insufficiency

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OBJECTIVE: To examine the feasibility and significance of ^{13}C -Hiolein breath test in evaluating chronic pancreatitis-related exocrine insufficiency and efficacy of enzyme treatment.

METHODS: The ^{13}C -Hiolein breath test was used in 8 healthy volunteers (group 1), 8 chronic pancreatitis (CP) patients without steatorrhea (group 2), and 8 CP patients with steatorrhea (group 3). To evaluate the function of pancreatic exocrine, $^{13}\text{CO}_2$ was determined following ^{13}C -Hiolein diet. The ^{13}C -Hiolein test was repeated in group 3 after enzyme supplement therapy.

RESULTS: Administration of ^{13}C -Hiolein diet resulted in significantly higher cumulative percent dose of ^{13}C recovery per 6 h (cPDR/6 h) and maximal PDR (PDR_{peak}) in the healthy controls (group 1) than the CP patients with steatorrhea (group 3) ($11.22\% \pm 1.22\%$ and $6.11\% \pm 0.59\%$ vs. $2.87\% \pm 0.73\%$ and $1.53\% \pm 0.36\%$, respectively, both $P < 0.01$). In the CP patients with steatorrhea (group 3), a repeated test after enzyme supplementation therapy showed a significant elevation of both cPDR/6 h and PDR_{peak} ($9.03\% \pm 0.84\%$ and $2.33\% \pm 0.47\%$, both $P < 0.01$ compared with those before enzyme treatment), but cPDR/6 h remained significantly lower than that in the healthy volunteers (group 1, $P < 0.05$). Both cPDR and PDR_{peak} in the CP patients without steatorrhea (group 2) were similar to those in the healthy controls (group 1, both $P > 0.05$).

CONCLUSION: The results of ^{13}C -Hiolein breath test well reflect fat metabolism status in CP patients, and the test can be used to monitor the efficacy of pancreatic enzymes therapy.

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Key words: pancreatitis; pancreatic exocrine; breath test; Hiolein

Introduction

Advanced chronic pancreatitis (CP) often results in an impaired pancreatic exocrine function. Many current tests for pancreatic exocrine function, such as secretincholecystokinin (CCK) and caerulein test are mostly used for research purposes, but are limitedly usable in clinic because of either being invasive, complicated to operate or

poorly stable.^[1,2] In this study, we examined pancreatic exocrine function using ^{13}C -Hiolein breath test to establish a simple, clinically operable examination.

Methods

Patients and controls

In this study normal volunteers and patients were involved. Control group (group 1) comprised eight normal volunteers (4 men and 4 women at age ranging from 26 to 67 years) who underwent routine physical and abdominal B ultrasound examinations to exclude gastrointestinal diseases, obesity, diabetes mellitus and thyroid diseases before this study. Group 2 consisted of 8 CP patients without stea-

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torrhea (4 men and 4 women, aged from 45 to 63 years). Group 3 included 8 CP patients with steatorrhea (6 men and 2 women at age of 49 to 66 years), and were given one pancreatic enzyme supplement tablet (Creon amylase 600 U, protease 800 U, lipase 1000 U) 3 times a day for 3 days before breath test. The diagnosis of CP was made based on a typical history of recurrent epigastric pain. Group 2 contained 2 patients who had pancreatic calcification and distension of the main pancreatic duct determined by computed tomography (CT), 3 patients who demonstrated formation of post-acute pancreatitis pseudocyst, and 3 patients who underwent surgery for pancreatic carcinoma but histological examination only showed evidence of inflammatory cell infiltration and fibrosis. In group 3, image examinations including B ultrasound, CT, endoscopic retrograde cholangiopancreatography (ERCP) demonstrated pancreatic atrophy, swelling of the main pancreatic duct and formation of pseudocyst in 6 patients, and the other two had irregular distension and constriction of the main pancreatic duct.

¹³C-Hiolein test

The reagent for ¹³C-Hiolein test purchased from Proto Corporation, Alberta, Canada was a mixture of ¹³C-labelled long chain triglycerides containing 15 mg ¹³C, which was predominantly labelled in their branch chains. Its fatty acid composition is shown in Table 1.

Fecal examination

The feces of all CP patients were collected from multiple parts, speared on the slides, stained by Sudan III and examined microscopically. Orange

development was steatorrhea positive.

Test procedures

All subjects were given low fat diet for 3 days and 12-hour fasting before ¹³C-Hiolein breath test. The test was performed in early morning when the patients were in a rest status. It was initiated by examining the breath samples that were collected after expiring into the bottom of a tube for 4–8 seconds through a straw, and the tube lid was tightened, immediately before ¹³C-Hiolein meal, which was defined as 0 minute ¹³C enrichment, called 0 minute $\delta^{13}\text{C}$ ($\delta = \text{‰}$). A 113 g standard budding meal mixed with 3 ml Hiolein containing 15 mg ¹³C was then taken within 5 minutes. The patients were left fasted for 6 hours, allowed to drink a small amount of water after 6 hours, and returned to normal diet after 8 hours. Breath sample was collected as described above every half hour after the meal. $\delta^{13}\text{C}$ in each breath sample was determined by an isotope ratio mass spectrometer (AP manufacturer, UK).

Calculation and statistics

$\delta^{13}\text{C}$ in each breath sample was adjusted by an international standard, pee dee belemnite (PDB). $\delta^{13}\text{C}$ at each time point–0 minute $\delta^{13}\text{C}$ was defined the change of ¹³C at that time ($\Delta\delta^{13}\text{C}$). $\Delta\delta^{13}\text{C}(\text{‰}) = (R_{\text{sample}}/R_{\text{PDB}} - 1) \times 1000$, in which R_{sample} was ¹³C/¹²C in each breath sample and $R_{\text{PDB}} = 0.01123372$. ¹³C cumulative recovery (cPDR) and peak PDR (PDR_{peak}) were calculated according to CO₂ production (presumed to be 5 mmol/min/m²) and the body surface area determined by Haycock G method.^[3,4] The data were statistically analyzed by State 6 software for variance, and paired Student's *t* test.

Results

0 minute $\delta^{13}\text{C}$ in all subjects were ranged from –30.27‰ to –28.29‰ (–29.23‰±0.42‰). The mean base $\delta^{13}\text{C}$ in the normal controls (group 1), –29.28‰±0.35‰, was similar to that in the chronic pancreatitis groups (groups 2 and 3),

Table 1. Fatty acid composition of Hiolein

Fatty acid	Name	Proportion (%)
18:1 ω-6	Oleic acid	50.6
16:0 ω-6	Palmitic acid	16.9
18:2 ω-6	Linolenic acid	19.6
18:3 ω-3	α-linolenic acid	3.0
18:0 ω-6	Stearic acid	2.3
16:1 ω-9	Palmitoleic acid	1.8
16:2 ω-6	No common name	1.4
16:3 ω-6	No common name	1.2

Table 2. Hiolein breath test in different groups

Group	cPDR (% dose/6 h)	PDR _{peak} (%)	Time(Peak)/h
1	11.22 ± 1.22	2.87 ± 0.73	5.90 ± 1.17
2	10.34 ± 0.92	2.60 ± 0.40	5.57 ± 1.05
3	6.11 ± 0.59*	1.53 ± 0.36*	5.78 ± 1.20

* compared to group 1, $P < 0.01$.

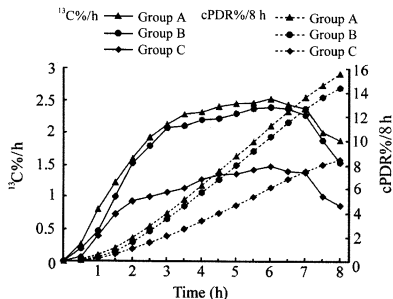


Fig. 2. Time courses for the excretion of ^{13}C and cumulative percent dose of ^{13}C recovery in breath over 8 h following of Hiolein breath test.

$-29.18\% \pm 0.33\%$ ($P > 0.05$). The $^{13}\text{CO}_2$ output in group 1 significantly increased at 0.5 hours after Hiolein meal, continuously increased, reached peak PDR at 5–6.5 hours (average 5.9 hours), and gradually declined thereafter (Fig.). The Hiolein fatty acid metabolism appeared to show a parabola style curve. No difference was observed in the required time to reach PDR_{peak} (Fig.).

Both ^{13}C cPDR/6 h and PDR_{peak} in the CP patients with steatorrhea (group 3) was markedly lower than those in groups 1 and 2 (both $P < 0.01$), but there was no significant difference of either ^{13}C cPDR/6 h or PDR_{peak} between groups 1 and 2 ($P > 0.05$, Table 2).

^{13}C cPDR/6 h and PDR_{peak} were $9.03\% \pm 0.84\%$ and $2.33\% \pm 0.47\%$, respectively in patients of group 3 three days after enzyme supplement therapy, which were significantly higher than those before the therapy ($P < 0.01$); however, ^{13}C cPDR/6 h remained lower than that in the normal controls (group 1), suggesting replacement of enzyme may not be sufficient.

Discussion

Because of calcification, fibrosis, atrophy and irregular distension or constriction of the pancreatic duct, most CP patients have a considerable malabsorption of dietary fats due to pancreatic enzyme insufficiency leading to impaired lipolysis. The patients, however, catch doctor's attention with the clinical symptoms of epigastric discomfort and steatorrhea after a fatty diet usually does not appear until the pancreatic lipase output markedly reduces to less than 10% of normal levels, since the pancreas possess a powerful capacity of compensation. The earliest alteration is usually the secretion reduction of pancreatic lipases; therefore, fecal fatty tests have long become a relatively-specific method for the diagnosis of CP. Sudan III staining is a classical qualitative test, and van de Kamer, a quantitative test (collection of 72 hour feces on fixed ingestion of fats at 100 g/d). However, both tests have not been well accepted by either doctors or patients because of time-consuming, unpleasant and complicated procedures, etc.^{1,5]}

Breath test for fat malabsorption using ^{13}C -labelled fatty acid as the substrate for lipolysis has been shown to be valid and diagnostically useful. When the ^{13}C -labelled fatty acids are taken, the absorbed substrates undergo β -oxidation and hydrolysis by duodenal lipases, emulsification by bile, absorption by intestinal mucosa, and ultimate expiration as CO_2 via breath. The unabsorbed ^{13}C -labelled fats are excreted via the feces. Vantrappen et al.^{13]} showed excellent correlations of lipase activities in the duodenum with ^{14}C -cRDP/6 h output in a mixed ^{14}C -triglyceride (^{14}C -MTG) breath test, as well as with duodenal amylase and alfapsin activities following cholecystokinin (CCK) stimulation. These data suggest that absorption and metabolism of fatty acids in human body can be indirectly assessed by measuring $^{14}\text{CO}_2$ in breath. John et al.^{16]} however, reported that ^{13}C excretion via the breath was decreased, but ^{13}C -labelled substrate in the feces was increased in patients with liver diseases, intestinal mucosal disorders or insufficiency in pancreatin, using ^{13}C oleic acids. These observations suggest that the results of fatty

acid breath test is influenced by many factors in lipid metabolism, including emulsification of bile, secretion of pancreatic lipases and integrity of intestinal mucosa.

The commonly used substrates include isotope-labelled MTG, palmitic and triolein. Hiolein used in this study was a mixture of uniformly ^{13}C -labelled long chain triacylglycerols obtained from algae with the fatty acid compositions of oleic, palmitic acid and linoleic acid, which were similar to those in normal foods. It therefore may well reflect the lipid absorption and metabolism function.^[7] The advantages of the Hiolein breath test include: (1) noninvasive; (2) simple, and well accepted by patients and doctors; (3) relatively specific to diagnosis of pancreatic exocrine insufficiency if hepatic biliary and intestinal disorders are ruled out; (4) prevention of environment contamination by ^{14}C by replacement with stable ^{13}C . However, its disadvantages are obvious: (1) low sensitivity to diagnose CP. Thus, in 20 CP patients, only 10 with steatorrhea, i.e., insufficient pancreatic exocrine, demonstrated abnormal $^{13}\text{CO}_2$; (2) requirement of special instrument.

In this study, we demonstrated the improvement of ^{13}C -Hiolein breath test results in the CP patients with steatorrhea who were given 3-day supplement therapy with high dose of lipases, which further enhanced the specificity of this test in assessing the pancreatic exocrine function. In addition, ^{13}C did not alter following supplement of pancreatic lipases if the CD patients with steatorrhea suffered from damage of intestinal mucosa, such as Chron's disease or α -anti trypsin deficiency-related hepatic disorder.^[8] These observations indicate the higher accuracy in diagnosis of pancreatic exocrine insufficiency after Hiolein breath test for twice.

Our study revealed that PDR reached its peak at about 6 hours, suggesting that the collections of breath samples can be reduced twice, i. e., before meal and 6 hours after meal, which would simplify the test procedures and be more acceptable by applicants.

In conclusion, the ^{13}C Hiolein breath test is able to effectively assess the pancreatic exocrine. Re-test after supplement therapy of pancreatic lipases improves its diagnostic accuracy in CP patients with steatorrhea. Its specificity and sensitivity remain to be determined by more studies.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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