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Influence of chronic ethanol consumption on extra-pancreatic secretory function in rat

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Abstract

Background: The usefulness of the typical direct methods involving duodenal intubation, such as the secretin and secretin–cholecystokinin tests, in the diagnosis of exocrine pancreatic dysfunction is widely accepted. However, these diagnostic tests tend to be avoided because of their technical complexity and the burden on patients. Recently, a simple breath test was developed for assessment of exocrine pancreatic function employing 13C-dipeptide [i.e., benzoyl-L-tyrosyl-[1-13C] alanine (Bz-Tyr-Ala)]. Although alcohol abuse causes pancreatic damage in humans, this has been unclear in rats. **Aims**: The aim of the study is to evaluate the effect of ethanol exposure beginning at an early age on extra-pancreatic secretory function in rats. **Materials and Methods:** Twelve female rats of the F344 strain aged 12 months were used. Seven rats were fed on a commercial mash food with 16% ethanol solution (Japanese Sake) as drinking-fluid since at 29 days of age (ethanol group). The remaining five rats were fed on a nutrient-matched isocaloric diet with water as drinking-fluid (control group). After 24-hr fasting, rats are orally administrated 1cc of water containing sodium 13C-dipeptide (5 mg/kg) and housed in an animal chamber. The expired air in the chamber is collected in a breath-sampling bag using a tube and aspiration pump. The 13CO2 concentration is measured using an infrared spectrometer at 10-min interval for 120 min and expressed as delta per mil. **Results**: The breath 13CO2 level increased and peaked at 20 min in both two groups. In general, 13CO2 excretion peaked rapidly and also decreased sooner in ethanol rats than in control rats. The mean value of the maximal 13CO2 excretion is 34.7 per mil in ethanol rats, greater than in control rats (31.4 per mil), but the difference did not reach the statistically significance. **Conclusion**: Chronic ethanol feeding beginning at an early age does not affect extra-pancreatic secretory function in rats.

Keywords: Acetate oxidation; ^{13}C-acetate breath test; ovariectomy; ageing.

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Introduction

Chronic ethanol consumption leads to fatty liver in the absence of nutritional deficiencies [1-3]. Although alcohol abuse causes pancreatic damage in humans, it has been unknown whether ethanol-feeding rats develop exocrine pancreatic dysfunction. Pancreatitis, acute and chronic, is closely associated with alcohol abuse, but symptomatic pancreatitis develops in only 10–20% of persons who abuse alcohol for long periods [4, 5]. In addition, no satisfactory model of chronic pancreatitis induced by ethanol alone has been established [6, 7]. Although alcohol abuse causes pancreatic damage in humans, this has been unclear in rats. Therefore, we put forward the hypothesis that

chronic ethanol feeding beginning at an early age might cause chronic pancreatitis or affect the exocrine pancreatic function.

On the other hand, the usefulness of the typical direct methods involving duodenal intubation, such as the secretin and secretin–cholecystokinin tests, in the diagnosis of exocrine pancreatic dysfunction is widely accepted. However, these diagnostic tests tend to be avoided because of their technical complexity and the burden on patients. Recently, a simple breath test was developed for assessment of exocrine pancreatic function employing ^{13}C-dipeptide [i.e., benzoyl-L-tyrosyl-[1-^{13}C] alanine (Bz-Tyr-Ala)] [8]. The aim of the study is to evaluate the effect of ethanol exposure beginning at an early age on extra-pancreatic secretory function in rats using this new non-invasive breath test.

Materials and Methods

Animals and laboratory

A total of 12 F344/DuCrj rats aged four weeks were purchased from CLEA Japan Inc. (Tokyo, Japan). Animals were housed in a quiet and temperature- and humidity-controlled room (22-24℃ and 50-60%, respectively) individually. The scheme of study design is demonstrated in Fig.1. Seven rats were fed on a commercial mash food with 16% ethanol solution (Japanese Sake) as drinking-fluid since at 29 days of age (ethanol group). They drank a 16% ethanol solution with net ethanol 9.7g/kg body weight on average. The remaining five rats were fed on a nutrient-matched isocaloric diet with water as drinking-fluid (control group). The weight of the rats was recorded every day, and daily water and food intake was measured between 900 and 1000 h. All procedures were approved by the Institutional Animal Care and Use Committee of Toho University, Tokyo, Japan.

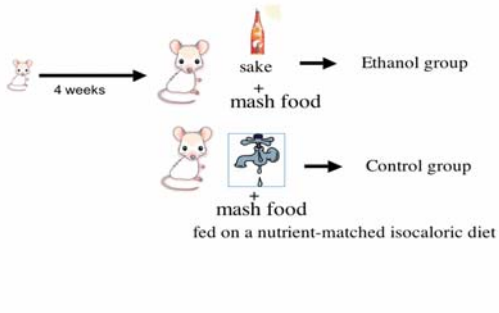

Fig.1 The scheme of study design. Seven rats were fed on a commercial mash food with 16% ethanol solution (Japanese Sake) as drinking-fluid since at 29 days of age (ethanol group).

Breath test

After 24-hr fasting, ^{13}C-dipeptide breath test was performed using the system for monitoring the ^{13}CO2 levels in expired air from small animals reported by Uchida et al [9]. After the rats were placed in the chamber for 10 minutes, 1200 mL of expired air was collected into the sampling bag as a baseline. Next, rats were orally administrated 1 mL of water containing sodium ^{13}C-dipeptide (5 mg/kg) and housed in an animal chamber. The expired air in the chamber is collected in a breath-sampling bag using a tube and aspiration pump at 10-min interval for 120 min. The ^{13}CO2 concentration is measured using an infrared spectrometer (Ubit IR-300; Ohtsuka Pharmaceutical Co., Ltd., Japan) and expressed as delta per mil (Δ‰). The maximum concentration (Cmax; ‰) and the time taken to reach the maximum concentration (Tmax; min) were used to evaluate the results of breath test.

Statistical analysis

Results are reported as means (SD) unless otherwise indicated. The maximum values of ^{13}CO2 excretion between two groups were compared at each age using Student's *t* test. Repeated measures ANOVA were used to examine between group differences in breath ^{13}CO2 excretion, since we had measurements for all rats at all nine time points. All analyses were done by the Statistical Package (JMP v 6.0 in Japanese edition).

Results

The average values of the ^{13}CO2 excretion at each sampling point after administration of ^{13}C-dipeptide are shown in Fig. 2. The breath ^{13}CO2 level increased rapidly and peaked at 20 min in both two groups, and decreased with time thereafter. In general, ^{13}CO2 excretion peaked more rapidly and also decreased sooner in ethanol rats than in control rats. Cmax is 36.5 ± 11.1 ‰ in ethanol rats, greater than in control rats (34.3 ± 13.8 ‰), but the difference did not reach the statistically significance (Fig.3). Tmax is 27.1 ± 4.9 min in ethanol rats and 28.0 ± 8.4 min in control rats (Fig.4). Similarly to Cmax, there was no significant difference in Tmax between the two groups.

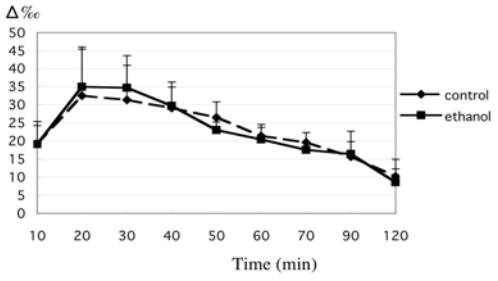

Fig. 2 The time course of 13CO2 excretion in ethanol-feeding rats and controls. The vertical axis is 13CO2 concentration expressed as delta per mil (Δ‰). The expired air is collected in a breath-sampling bag at 10-min interval after ingestion of 13C-dipeptide. 13CO2 excretion peaked more rapidly and decreased sooner in ethanol rats than in control rats.

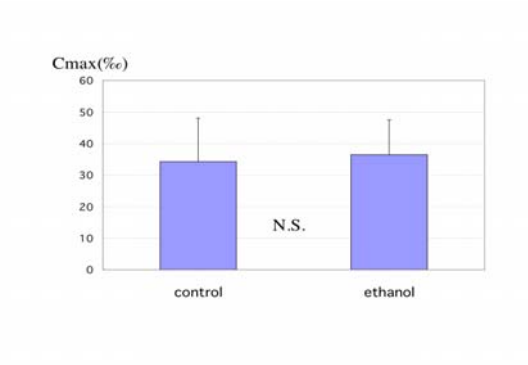

Fig. 3 Bars indicate the maximum concentration (Cmax; ‰) of 13CO2 in the expired air, which is 36.5 ± 11.1 ‰ in ethanol rats, greater than in control rats (34.3 ± 13.8 ‰).

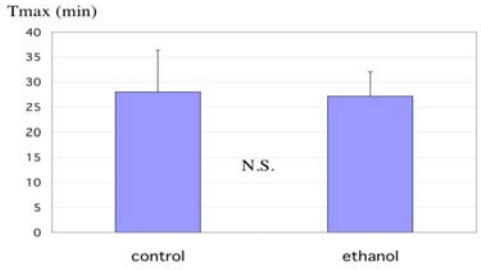

Fig. 4 Bars indicate the time taken to reach the maximum concentration (Tmax; min). Tmax is 27.1 ± 4.9 min in ethanol rats and 28.0 ± 8.4 min in control rats. The difference did not reach the statistically significance.

Discussion

The feasibility of administering an oral dose of a ^{13}C-substrate and procuring metabolic or diagnostic information from its metabolism and conversion to ^{13}CO2 is attractive due to its noninvasive nature. With the increase use of mass spectrometry and stable isotopes, a various kind of ^{13}C-substrates have been proposed to evaluate gastrointestinal function, including gastrointestinal motility, digestive function, absorptive function, liver function, and metabolism of nutrients. ^{13}C breath tests provide precise evaluations of the presence or absence of etiologically significant changes in metabolism due to a specific disease or the lack of a specific enzyme. The enzyme-substrate interaction results in the release of ^{13}CO2 in the expired breath. ^{13}C-urea breath test for the detection of Helicobacter pylori typifies such breath tests. ^{13}C-phenylalanine breath test, ^{13}C-α-ketoisocaproic acid breath test, and ^{13}C-galactose breath test have been used for the evaluation of liver function by measuring the enzyme activities [10]. The physician can obtain valuable diagnostic information on the enzyme activities by distinguishing between two groups on the basis of the recovery of ^{13}CO2 from the ingested ^{13}C-substrate.

On the other hand, the assessment of exocrine pancreatic function is difficult even by using ^{13}C breath tests. Since exocrine pancreatic function has an important role on digestive process of various nutrients, various ^{13}C-substrates have been used for the assessment of exocrine pancreatic function, including ^{13}C-trioctanoin [11], ^{13}C-triolein [12], ^{13}C-mixed triglycerides [13], ^{13}C-egg white protein [14], or ^{13}C-starch [15]. These breath tests take up much of time up to several hours and the patient have to be asked to avoid both breakfast and lunch. Therefore, ^{13}C breath test has not been accepted as a screening tool for the assessment of exocrine pancreatic function in a clinical setting. Recently, a simple breath test was developed for assessment of exocrine pancreatic function employing ^{13}C-dipeptide [i.e., benzoyl-L-tyrosyl-[1-^{13}C] alanine (Bz-Tyr-Ala)] [8] in which the study period was only 90 min. Bz-Tyr-Ala is cleaved into benzoyl-L-tyrosyl and [1-^{13}C] alanine by carboxypeptidase in pancreatic juice in the duodenum, and [1-^{13}C] alanine is absorbed and reaches the liver through the bloodstream, and is metabolized to release ^{13}CO2 in the exhaled breath [8]. The newly developed test has to be compared to a gold standard test or an established method of evaluating exocrine pancreatic function. Ishii et al [8, 16] also compared the results of ^{13}C-dipeptide breath test to those of pancreatic juice volume and N-benzoyl-L-tyrosyl-p-aminobenzoic acid (BT-PABA) test, resulting in a close association between these tests in human.

There have been few experimental animal studies using ^{13}C breath test because it is difficult to collect breath samples of small animals. Since the system for monitoring the ^{13}CO2 levels in expired air from small animals was developed by Uchida et al [9] in 2005, ^{13}C breath test has been used in experimental animal studies. Similarly to human studies, Uchida et al [17] also reported that exocrine pancreatic function can be evaluated using ^{13}C-dipeptide breath test in rats. Then the present study using ^{13}C-dipeptide breath test was proposed to evaluate the relationship between long-term ethanol consumption beginning at an early age and exocrine pancreatic function in rats. It is because chronic alcohol consumption has been considered as one of the major causes of chronic pancreatitis but less than 10% of chronic clcoholics develop chronic pancreatitis [4]. Our poor understanding of the pathophysiological events leading to the onset of chronic pancreatitis results from the fact that most patients with the disease are identified only after the early phases of the disease have passed. Clinical studies focusing on mechanisms responsible for the onset of chronic pancreatitis are not possible. To overcome this problem, numerous attempts at developing animal models of chronic pancreatitis have been made, but the goal of developing a good animal model of the disease has not been achieved. Therefore, the ethanol-feeding rats are used in the present study to evaluate the association between long-term alcohol consumption and developing chronic pancreatitis.

Although gastroenterologists frequently encounter patients with chronic pancreatitis, which is responsible for 86000 annual admission in United States [18], the diagnosis of chronic pancreatitis is suspected on the basis of compatible signs and symptoms and most often confirmed by imaging studies because pancreatic histology is rarely

available to clinicians. However, radiographic examinations are insensitive and nonspecific, especially for early stage disease. Therefore, most physicians are often forced to rely on pancreatic function tests, which have the potential to detect damage to the pancreas that is less obvious and less advanced. The reference method for an early diagnosis of exocrine pancreatic insufficiency is the invasive secretin-pancreocymin test in which a big expenditure of costs, time, and manpower placing is required. This test is seldom performed even in authorized hospitals. In contrast, ^{13}C-breath tests are noninvasively performed and reflect the intraduodenal activities of pancreatic enzymes under physiological conditions. Until now, several variations of breath tests with 13C-substrates in fatty acid triglyceride have been developed to measure intraluminal fat digestion by pancreatic lipase [11-15]. The high time expenditure and the lack of standardization still limit the clinical use of these breath tests except for ^{13}C-dipeptide breath test.

In the present study, ^{13}CO2 excretion peaked rapidly and also decreased sooner in ethanol rats than in control rats, but the difference did not reach the statistical significance. This suggests that chronic ethanol feeding beginning at an early age does not affect extra-pancreatic secretory function in rats. Li J et al [19] reported that long-term alcohol consumption did not cause chronic pancreatitis but impaired exocrine pancreatic function using Wister rats fed diet containing 25% concentration of ethanol for 6 months. This study revealed an obvious decrease of CCK in both small intestine and pancreas after chronic ethanol intake. In contrast, some negative results were found in several studies of the effect of prolonged ethanol intake on exocrine pancreas [20, 21]. These discrepancies might be due to the differences in the functional test methods, duration of ethanol administration, or kinds of animals used.

Conclusion

Since the result of ^{13}C-dipeptide breath test reflects enzyme activity of carboxypeptidase in pancreatic juice, the fact that chronic ethanol feeding beginning at an early age does not affect the secretion of carboxypeptidase in a fasting state is developed in the present study. If pancreatic stimulation is performed before ^{13}C-dipeptide breath test, the result might be different. It is concluded that 12-month ethanol feeding beginning at an early age does not affect basal extra-pancreatic secretory function in rats.

Acknowledgement

Toshiyasu Watanabe, Tsunehiko Imai, and Yoshihisa Urita wrote the paper and contributed to acquiring data. Yasuyuki Miura and Naohiro Washizawa contributed to analyzing data. Masaki Sanaka, Nagato Shimada and Hitoshi Nakajima contributed to drafting the manuscript. Yoshiko Honda and Motonobu Sugimoto contributed to enhancing its intellectual content. We declare that there are not any potential conflicts of interest that are relevant to the manuscript.

References

1. Rao GA, Larkin EC. Nutritional factors required for alcoholic liver disease in rats. J Nutr 1997; 127(5 Suppl): 896S-898S
2. Mendenhall CL, Rouster SD, Roselle GA, Grossman CJ, Ghosn S, Gartside P. Impact of chronic alcoholism on the aging rat: changes in nutrition, liver composition, and mortality. Alcohol Clin Exp Res 1993; 17: 847-853.
3. Ellingson JS, Janes N, Taraschi TF, Rubin E. The effect of chronic ethanol consumption on the fatty acid composition of phosphatidylinositol in rat liver microsomes as determined by gas chromatography and 1H-NMR. Biochim Biophys Acta 1991; 1062: 199-205.
4. Sakorafas GH, Tsiotou AG. Etiology and pathogenesis of acute pancreatitis: current concepts. J Clin Gastroenterol 2000; 30:343-356.
5. Schenker S, Montalvo R. Alcohol and the pancreas. Recent Dev Alcohol 1998; 14:41-65.
6. Deng X, Wang L, Elm MS, Gabazadeh D, Diorio GJ, Eagon PK, Whitcomb DC. Chronic alcohol consumption accelerates fibrosis in response to cerulein-induced pancreatitis in rats. Am J Pathol 2005; 166:93-106.
7. Perides G, Tao X, West N, Sharma A, Steer ML. A mouse model of ethanol dependent pancreatic fibrosis. Gut 2005; 54:1461-1467.
8. Ishii Y, Kohno T, Ito A, Suzuki S, Kohno T, Takayama T, Asai S. Measurement of extra-pancreatic secretory function by 13C-dipeptide breath test. Transl Res 2007; 149:298-303.
9. Uchida M, Endo N, Shimizu K. Simple and noninvasive breath test using 13C-acetic acid to evaluate gastric emptying in conscious rats and its validation by metoclopramide. J Pharmacol Sci 2005; 98: 388-395
10. Perri F, Marras RM, Ricciardi R, Quitadamo M, Andriulli A. ^{13}C-breath tests in hepatology (cytosolic liver function). Eur Rev Med Pharmacol Sci 2004; 8: 47-49.
11. Watkins JB, Schoeller DA, Klein PD, Ott DG, Newcomer AD, Hofmann AF. 13C-trioctanoin: a nonradioactive breath test to detect fat malabsorption. J Lab Clin Med 1977; 90: 422-430.
12. Watkins JB, Klein PD, Schoeller DA, Kirschner BS, Park R, Perman JA. Diagnosis and differentiation of fat malabsorption in children using 13C-labeled lipids: trioctanoin, triolein, and palmitic acid breathe tests. Gastroenterology. 1982; 82: 911-917.
13. Vantrappen GR, Rutgeerts PJ, Ghoos YF, Hiele MI. Mixed triglyceride breath test: a noninvasive test of pancreatic lipase activity in the duodenum. Gastroenterology 1989; 96: 1126-1134.
14. Evenepoel P, Hiele M, Geypens B, Geboes KP, Rutgeerts P, Ghoos Y. 13C-egg white breath test: a non-invasive test of pancreatic trypsin activity in the small intestine. Gut 2000; 46: 52-57.
15. Hiele M, Ghoos Y, Rutgeerts P, Vantrappen G. Starch digestion in normal subjects and patients with pancreatic disease, using a 13CO2 breath test. Gastroenterology 1989; 96: 503-509.
16. Ishii Y, Kohno T, Ito A, Suzuki S, Kohno T, Takayama

T, Asai S. Evaluation of pancreatic exocrine secretion using 13C-dipeptide (benzoyl-L-tyrosyl-[1-(13)C]alanine) breath test: focusing on pancreatoduodenectomy cases. Pancreas 2007; 35: 313-319.
17. Uchida M, Mogami O. Usefulness of breath test for evaluating pancreatic exocrine function using N-benzoyl-L-tyrosyl-1-13C-L-alanine sodium in non-invasive and conscious rats. Biol Pharm Bull 2008; 31: 785-788.
18. Kozak LJ, Owings MF, Hall MJ. National Hospital Discharge Survey: 2002 annual summary with detailed diagnosis and procedure data. Vital Health Stat 13. 2005; 158: 1-199.
19. Li J, Guo M, Hu B, Liu R, Wang R, Tang C. Does chronic ethanol intake cause chronic pancreatitis?: evidence and mechanism. Pancreas. 2008; 37: 189-195.
20. Grönroos JM, Aho HJ, Meklin SS, Hakala J, Nevalainen TJ. Pancreatic digestive enzymes and ultrastructure after chronic alcohol intake in the rat. Exp Pathol. 1988; 35: 197-208.
21. Norton ID, Apte MV, Lux O, Haber PS, Pirola RC, Wilson JS. Chronic ethanol administration causes oxidative stress in the rat pancreas. J Lab Clin Med. 1998; 131: 442-446.