카르니틴이 비알코올성 지방간 질환에서 말초혈액 미토콘드리아 DNA 단위 반복수와 간기능에 미치는 영향

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Effects of Carnitine on Peripheral Blood Mitochondrial DNA Copy Number and Liver Function in Non-Alcoholic Fatty Liver Disease

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Background/Aims: Functional and anatomical abnormalities of mitochondria play an important role in developing steatohepatitis. Carnitine is essential for enhanced mitochondrial beta oxidation through the transfer of long-chain fatty acids into the mitochondria. We examined the impact of carnitine complex on liver function and peripheral blood mitochondria copy number in NAFLD patients. Methods: Forty-five NAFLD patients were enrolled. Patients were categorized into the carnitine complex-administered group and control group. Before and 3 months after drug administration, a liver function test and peripheral blood mitochondrial DNA and 8-oxo-dG quantitative analysis were conducted. Results: In carnitine treatment group, ALT, AST, and total bilirubin were reduced after medication. There was no difference in AST, ALT, and total bilirubin between before and after treatment in control group. In carnitine group, peripheral mitochondrial DNA copy number was significantly increased from 158.8±69.5 copy to 241.6±180.6 copy (p=0.025). While in control group the mitochondrial copy number was slightly reduced from 205.5±142.3 to 150.0±109.7. 8-oxo-dG level was also tended to decrease in carnitine group (p=0.23) and tended to increase in control group (p=0.07). Conclusions: In NAFLD, the carnitine improved liver profile and peripheral blood mitochondrial DNA copy number. This results suggest that carnitine activate the mitochondria, thereby contributing to the improvement of NAFLD. (Korean J Gastroenterol 2010;55:384-389)

Key Words: Nonalcoholic fatty liver disease; Mitochondria; Carnitine

Introduction

Non-alcoholic fatty liver disease (NAFLD) is reported to be closely associated with obesity, insulin resistance. Also, NAFLD is related with various systemic complications such as cardiovascular, renal, and metabolic disease regardless of obesity and insulin resistance. However a distinctive pathogenesis has not yet been reported. The development of NAFLD is known

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to be associated with mitochondrial disorders. Mitochondrial disorders associated with NAFLD include structural lesions, mitochondrial DNA copy number reduction, reduced mitochondrial respiratory chain (MRC), and mitochondria β-oxidation disorders. In certain researches, when administered with the drug 4,4′-diethylaminoethoxyhexestrol aimed at inhibiting the mitochondria respiratory chain (MRC) activity and mitochondria β-oxidation, it resulted in increased mitochondrial formation of reactive oxygen species and caused lipid peroxidation in rats. Also, according to electron microscope findings of liver tissues in mouse model for insulin resistance. In diabetes similar to NAFLD, peripheral serum tumor necrosis factor alpha, body mass index, and insulin resistance. In diabetes similar to NAFLD, peripheral serum tumor necrosis factor alpha, body mass index, and insulin resistance. In diabetes similar to NAFLD, peripheral serum tumor necrosis factor alpha, body mass index, and insulin resistance.

Wu et al. reported glycyrhrizin reduced hepatic lipotoxicity by stabilizing the integrity of lysosomes and mitochondria. This suggests the restoration or protection of mitochondrial function is one of therapeutic option for NAFLD. Several anti-oxidative scavengers such as Coenzyme Q₁₀, carnitine, vitamins, and n-3-polysaturated fatty acid suggested modulate mitochondrial beta oxidation and function. L-carnitine is a cofactor of carnitine palmitoyltransferase 1 (CPT1), and L-carnitine in hepatocytes modulate hepatic CPT1A activity. Recent data suggested that the overexpression of CPT1 maintain mitochondrial function and ameliorate lipid induced insulin resistance through acceleration of β-oxidation. Carnitine was involved in several physiological roles: Carnitine acts as free radical scavenger and essential for the transfer of long-chain fatty acids into the mitochondria.

This study aimed to define the effects of carnitine on liver functions and peripheral blood mitochondrial DNA copy number in NAFLD patients.

Materials and Methods

1. Patients

Forty-five NAFLD patients who visited the gastroenterology department with an increase in AST/ALT were enrolled. All patients had fatty liver on abdominal sonography and allocated by non-randomized and open labeled manner. The definition of NAFLD was as follows (i) subjects with an alcohol consumption ≤20 g/day, (ii) subjects who were not taking drugs such as any herbal medication, amiodarone, methotrexate, synthetic estrogens, nucleoside analogues and glucocorticoids within three months, (iii) negative study for viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, drug-induced liver disease, and thyroid disease (hepatitis B surface antigen, anti-HCV antibody, anti-nuclear antibody, anti-mitochondrial antibody, thyroid function test).

The patients were categorized into the carnitine complex (Godex, Hanseo pharm. Co., Ltd, Seoul, Korea) administered group and the control group. Carnitine group took carnitine with a dose of 600 mg/day. Before and 3 months after drug administration, with patients diagnosed as NAFLD, liver function tests such as albumin, total bilirubin, AST, ALT, γ-GTP, and as well as mitochondrial DNA content were conducted. The institutional review boards of hospital approved the study.

2. Measurement of peripheral blood mitochondrial DNA

Before and after each drug administration, 5 mL of peripheral venous blood was taken, put in the EDTA tube, and immediately kept at −80°C. At room temperatures, with the blood centrifugation, DNA was extracted using QIAamp DNA Mini Kit (QIAGEN, Co., Ltd, Düsseldorf, Germany), and peripheral blood mitochondrial DNA was measured using real time PCR. Probes for GAPDH and mtDNA were made, and the 5’ end and the 3’ end were marked with 5-carboxyfluorescein (FAM) reporter and 6-carboxy-tetramethyl-rhodamine ( TAMRA) quencher, respectively. mtDNA was made to undergo PCR, the PCR mixture, totaling 15 μL, consisted of TaqMan Universal PCR Master Mix (ABI, USA) 7.5 μL, primer forward (5 pmol/μL) 1.5 μL, primer reverse (5 pmol/μL) 1.5 μL, probe (1 pmol/μL) 1.5 μL, distilled water 0.5 μL, and DNA 2.5 μL. PCR was conducted using the ABI 7500 Real-Time PCR System, with the amplification environment involving 2 minutes at 50°C, one minute 40-time repetition at 60°C. To analyze the content of mitochondrial DNA copy number, Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) amplification was used. Primers and probes used are as follows: GAPDH forward primer; CCA GGT GGT CTC CTC TGA CT T C, GAPDH