PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 110-113
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Changes of plasma fasting carnitine ester profile in patients with ulcerative colitis

Judit Bene, Katalin Komlósi, Viktória Havasi, Gábor Talián, Beáta Gasztonyi, Krisztina Horváth, Gyula Mózsik, Béla Hunyady, Béla Melegh, Mária Figler

Judit Bene, MTA PTE Clinical Genetics Research Group of Hungarian Academy of Sciences at the University of Pécs, Pécs, Hungary
Judit Bene, Katalin Komlósi, Viktória Havasi, Gábor Talián, Béla Melegh, Department of Medical Genetics and Child Development, University of Pécs, Pécs, Hungary
Beáta Gasztonyi, Krisztina Horváth, Gyula Mózsik, Béla Hunyady, Mária Figler, 1st Department of Medicine, School of Medicine, University of Pécs, Pécs, Hungary
Supported by the grant of Ministry of Health, No. ETT 325/2003 and 595/2003; the grant of Hungarian Science Foundation, No. OTKA T 35026 and T 49589; and from the National grant No. NKFP-4/005/2002
Correspondence to: Dr Béla Melegh, Professor of Medical Genetics and Pediatrics, Department of Medical Genetics and Child Development, University of Pécs, H-7624 Pécs, Szigeti 12, Hungary. bela.melegh@aok.pte.hu
Telephone: +36-72-536427 Fax: +36-72-536427
Received: 2005-05-23 Accepted: 2005-06-18

Abstract

AIM: To determine the plasma carnitine ester profile in adult patients with ulcerative culitis (UC) and compared with healthy control subjects.

METHOD: Using ESI triple quadrupole tandem mass spectrometry, the carnitine ester profile was measured in 44 patients with UC and 44 age- and sex-matched healthy controls.

RESULTS: There was no significant difference in the fasting free carnitine level between the patients with UC and the healthy controls. The fasting propionyl- (0.331 ± 0.019 *vs* 0.392 ± 0.017 μmol/L), butyryl- (0.219 ± 0.014 *vs* 0.265 ± 0.012), and isovalerylcarnitine (0.111 ± 0.008 *vs* 0.134 ± 0.008) levels were decreased in the UC patients. By contrast, the level of octanoyl- (0.147 ± 0.009 *vs* 0.114 ± 0.008), decanoyl- (0.180 ± 0.012 *vs* 0.137 ± 0.008), myristoyl- (0.048 ± 0.003 *vs* 0.039 ± 0.003), palmitoyl- (0.128 ± 0.006 *vs* 0.109 ± 0.004), palmitoleyl- (0.042 ± 0.003 *vs* 0.031 ± 0.002) and oleylcarnitine (0.183 ± 0.007 *vs* 0.163 ± 0.007; $P < 0.05$ in all comparisons) were increased in the patients with UC.

CONCLUSION: Our data suggest selective involvement of the carnitine esters in UC patients, probably due to their altered metabolism.

Key words: Carnitine; Carnitine ester profile; Ulcerative colitis

Bene J, Komlósi K, Havasi V, Talián G, Gasztonyi B, Horváth K, Mózsik G, Hunyady B, Melegh B, Figler M. Changes of plasma fasting carnitine ester profile in patients with ulcerative colitis. *World J Gastroenterol* 2006; 12(1): 110-113

http://www.wjgnet.com/1007-9327/12/110.asp

INTRODUCTION

Ulcerative colitis (UC) is a disorder of the idiopathic and chronic inflammation of the colonic mucosa. The etiology and the pathogenesis of the disease are yet unknown; a classic study on isolated colonic epithelial cells demonstrated decreased utilization of n-butyrate. Since the major energy sources of the epithelial cells of the distal colon are the short-chain fatty acids (SCFAs), these cells are able to metabolize other fuels, such as glucose and glutamine, only at a much lower rate[1]. SCFAs are generated from carbohydrates by bacterial degradation and they are readily absorbed by the colon and represent energy fuels for the colonocytes and other tissues, such as the skeletal muscle[2,3]. Patients with distal UC may have increased or moderately decreased stool SCFA concentrations, reflecting their altered absorption[4,5]. UC can, therefore, be regarded essentially as an SCFA oxidation failure-associated disease, where the energy deficiency is a primary event in the development of the disease[1].

L-carnitine plays an essential role in the energy metabolism, since it enables the transport of activated long-chain fatty acids (LCFA) as carnitine esters across the inner mitochondrial membrane. Moreover, it is able to form esters with several medium- and SGFAs of both endogenous and exogenous origins[6,7]. The impact of altered SCFA metabolism in UC prompted us to study the circulating carnitine ester profile in the UC patients.

MATERIALS AND METHODS

Patients

We examined 44 patients with UC (25 males, 19 females,

Table 1 Some major clinical and laboratory parameters in patients with ulcerative colitis and control subjects (mean ± SE)

Parameters	UC patients, n = 44	Controls, n = 44
Females/males	19/25	24/20
CRP (mg/L)	12.2 ± 4.5	2.6 ± 0.5
Albumin (g/L)	44.6 ± 0.7	50.2 ± 0.8
Iron (μmol/L)	16.1 ± 1.2	23.7 ± 1.6
Hb (g/dL)	131.3 ± 2.5	159 ± 1.2
MCV (L)	86.2 ± 1.2	94.8 ± 2.5
WBC (G/L)	7.6 ± 0.4	9.2 ± 0.6
BMI (kg/m^2)	24.6 ± 0.6	24.1 ± 0.5
PLT (G/L)	298.5 ± 13.5	228.3 ± 10.4
Both ileum and colon localization	5/44 (11.4%)	
Rectosigmoid localization only	8/44 (18.2%)	
Colon localization	31/44 (70.4%)	

mean age: 39.7 years, range: 17-65 years), and 44 carefully selected clinically healthy age-, sex-, weight-, and height-matched control subjects (20 males, 24 females, mean age: 37.0 years, range: 23-60 years). The control subjects did not receive any drug medication, while the UC patients were treated with either sulfasalazine or 5-aminosalicylic acid. We assumed that these drugs do not have any influence on the carnitine status since there were no such data available in the literature.

Diagnosis of the disease relied upon the history of the patients, clinical symptoms, negative stool examination for bacteria and parasites, and histologic results of rectal and/ or colonic biopsy. Exclusion criteria in both groups were as follows: secondary causes of colonic disease, systemic diseases, any malformations, evidence of intestinal bacterial infection, history or evidence of any inherited metabolic disease, hepatic or renal disease, and pregnancy. (Table 1) shows the clinical parameters of the UC patients.

The clinical and laboratory data were the results of measurements performed from sample aliquots of blood collected after an overnight fasting precisely between 08:00 a.m. and 08:30 a.m., both in the UC patients and in the healthy control subjects. This strict post-alimental time scheduling was introduced to prevent the diet or fasting time-induced dynamic changes of carnitine esters in the circulation[8].

Informed consent was obtained from each participant of the study and the study design was approved by the Departmental Ethics Committee.

Methods

Plasma albumin, iron, and C-reactive protein levels were determined by routine methods. The hemoglobin (Hb), mean corpuscular volume (MCV), white blood cells (WBC) and platelet (PLT) were measured by automated analysis (sysmex XE 2100, Japan). The body mass index (BMI) was calculated as body weight/height^2 (in kg/m^2).

Acylcarnitines were measured after derivatization as butyl esters using isotope dilution mass spectrometry method in a Micromass Quattro Ultima ESI triple-quadrupole mass spectrometer. The procedure was principally the method described previously[9]. Essentially, 10 μL of plasma was spotted and dried onto a filter paper and prepared by extraction with 200 μL of methanol containing internal deuterium-labeled standards (0.76 μmol/L [$^2H_3$] -free carnitine, 0.04 μmol/L [$^2H_3$] -propionylcarnitine, 0.04 μmol/L [$^2H_3$] -octanoylcarnitine and 0.08 μmol/L [$^2H_3$] -palmitoylcarnitine). After 20 min of agitation, the supernatant was evaporated to dryness under nitrogen at 40 ℃ and then 100 μL of 3 mol/L butanolic HCl was added. The solution was incubated at 65 ℃ for 15 min and evaporated to dryness again under nitrogen at 40 ℃ and re-dissolved in 100 μL of the mobile phase (acetonitrile:water 80:20). A total of 10 μL of sample aliquots were introduced to the ESI cone by using Waters 2795 HPLC system. The free carnitine and all acylcarnitines were determined by ESI-MS/MS analysis using positive precursor ion scan of m/z 85; scan range was 200-550 m/z. The capillary voltage was 2.50 kV, while the cone voltage was 55 V, and the collision energy was 25 eV. The flow rate was 100 μL/min and the total analysis time was 4 min per sample. Each sample was measured in triplicates starting with the injection step, and the results were the means of the three determinations.

Statistical analysis

Student's t test for unpaired samples was used for the statistical analysis. The values were expressed as mean ± SE, in three decimals for the carnitine esters with respect to the low levels in the case of the long-chain carnitine esters. $P < 0.05$ was considered statistically significant.

RESULTS

The plasma circulating carnitine ester profiles are shown in Table 2. The plasma level of free carnitine and acetyl carnitine did not differ between the UC patients and the controls. By contrast, significant decreases were observed in fasting propionyl-, butyryl-, and isovalerylcarnitine ester levels in UC patients as compared with the controls. The level of total short-chain carnitine esters was markedly lower in the patients with UC (9.855 ± 0.094 μmol/L) than in the healthy controls (11.003 ± 0.100 μmol/L, $P < 0.01$).

The levels of octanoyl-, and decanoylcarnitine were decreased in the healthy subjects. The levels of total medium-chain acylcarnitines were obviously higher in the UC patients (0.629 ± 0.007 μmol/L) than in the control subjects (0.548 ± 0.007 μmol/L, $P < 0.01$).

In the long-chain acylcarnitine group, the plasma levels of myristoyl-, palmitoyl-, palmitoleyl- and oleylcarnitine were significantly decreased in the healthy group. The levels of total long-chain carnitine esters were markedly higher in the patients with UC (0.596 ± 0.005 μmol/L) than in the controls (0.515 ± 0.009 μmol/L, $P < 0.01$).

In addition, the level of total carnitine esters was significantly decreased in the UC patients (11.080 ± 0.035 μmol/L) as compared with the healthy controls (12.066 ± 0.037 μmol/L, $P < 0.01$).

DISCUSSION

Carnitine [β-hydroxy-γ(trimethylamino) butyric acid]

Table 2 Plasma carnitine ester profiles in ulcerative colitis patients and controls (μmol/L, mean ± SE)

	UC patients, n = 44	Controls, n = 44
Free carnitine (C0)	31.595 ± 1.454	31.431 ± 1.042
Short-chain acylcarnitines		
Acetylcarnitine (C2)	9.164 ± 0.426	10.179 ± 0.461
Propionylcarnitine (C3)	0.331 ± 0.019^a	0.392 ± 0.017
Butyrylcarnitine (C4)	0.219 ± 0.014^a	0.265 ± 0.012
Isovalerylcarnitine (C5)	0.111 ± 0.008^a	0.134 ± 0.008
Tiglylcarnitine (C5:1)	0.030 ± 0.004	0.033 ± 0.002
Medium-chain acylcarnitines		
Hexanoylcarnitine (C6)	0.071 ± 0.006	0.081 ± 0.005
Octenoylcarnitine (C8)	0.147 ± 0.009^a	0.114 ± 0.008
Octenoylcarnitine (C8:1)	0.064 ± 0.005	0.062 ± 0.007
Decanoylcarnitine (C10)	0.180 ± 0.012^a	0.137 ± 0.008
Cecenoylcarnitine (C10:1)	0.113 ± 0.006	0.104 ± 0.008
Lauroylcarnitine (C12)	0.054 ± 0.003	0.050 ± 0.003
Long-chain acylcarnitines		
Myristoylcarnitine (C14)	0.026 ± 0.001	0.024 ± 0.001
Myristoleylcarnitine (C14:1)	0.048 ± 0.003^a	0.039 ± 0.003
Palmitoylcarnitine (C16)	0.128 ± 0.006^a	0.109 ± 0.004
Palmitoylcarnitine (C16:1)	0.042 ± 0.003^a	0.031 ± 0.002
Stearoylcarnitine (C18)	0.085 ± 0.003	0.079 ±0.003
Oleylcarnitine (C18:1)	0.183 ± 0.007^a	0.163 ± 0.007
Hydroxymyristoylcarnitine (C14OH)	0.007 ± 0.001	0.006 ± 0.001
Hydroxypalmitoylcarnitine (C16OH)	0.026 ± 0.002	0.023 ± 0.001
Hydroxypalmitoleylcarnitine (C16:1OH)	0.033 ± 0.002	0.029 ± 0.002
Hydroxyoleylcarnitine (C18:1OH)	0.018 ± 0.002^a	0.012 ± 0.001

^{a}P < 0.05 *vs* controls.

is known as a carrier for transporting activated LCFA into the mitochondrial matrix for β-oxidation. With this function the L-carnitine plays an essential role in the energy metabolism[6]. Moreover, the carnitine molecule is able to form esters with several medium- and short-chain fatty acids of both endogenous and exogenous origins[6,7]. The circulating carnitine ester spectrum can reflect affected cellular metabolism of the short-, medium-, and long-chain fatty acids. Therefore, the monitoring of the carnitine ester composition is a widespread tool for the diagnosis of several inborn errors of metabolism. Besides the complete metabolic blockage caused by the inherited lack of enzyme activities, influences on carnitine ester spectra may be the consequence of only partially affected flux of metabolites via the carnitine acyltransferases.

In the present study, significant decrease was found in the fasting plasma levels of propionyl-, butyryl-, and isovalerylcarnitine esters, leading to the decrease of SCFA carnitine esters. Although the pathogenesis of UC is still unknown, a widely accepted hypothesis focuses on the pivotal role of the diminished availability of SCFAs for the enteral cells. Normally, SCFAs are rapidly absorbed from the colon and have many properties, as they represent an energy source for colonocytes and if they are exported to other tissues. Moreover, they affect lipid metabolism, colonic mucosal blood flow, motility, and mucus secretion[2]. In the normal case, the major energy source of the epithelial cells of the distal colon derives from the metabolism of the SCFAs[10], which is impaired in UC[1]. In addition to the SCFA metabolism, the influenced coenzyme A esterification has been reported to be associated with UC[11]. In the cells, the fatty acyl moieties are transferred from coenzyme A to the beta hydroxyl group of the carnitine via the short-, medium, and long-chain carnitine acyltransferases[6]. These events separately or in combination, can explain the decrease of the circulating SCFA carnitine esters.

The circulating plasma carnitine profile is determined by the balance of the release and uptake mechanisms. Carnitine releases into the circulation by the liver primarily as acylcarnitine[12]. While in the hepatic vein, the ester proportion is relatively high, approximately half of the total carnitine is esterified. The actual ester pattern detected in the peripheral blood is a result of the uptake/release action of the peripheral tissues; and in a peripheral venous blood, much less (approximately 1/3-1/4 of the total carnitine) is esterified. Whereas, the decrease of the SCFA carnitine esters found in the UC patients could be explained as discussed earlier. Based on the current knowledge, it is much more difficult due to the limited nature of the data, to explain the opposite change of the medium- or long-chain carnitine esters. Only a few studies are available reporting alterations of fatty acid metabolism. However, the data are inconsistent, but suggest the involvement of LCFA metabolism in UC[13-15]. Further studies are required to clarify these issues.

After the positive results on topical SCFA treatment in UC[16], Gasbarrini *et al*[3] studied propionyl-L-carnitine administration as rectal irrigation and found that improved clinical picture and histological status of the bowel are improved. In the light of the current findings, the likely decreased tissue reserves could be corrected by administration of the drug and the positive outcome could be a consequence of the successful replacement therapy. Whether the already known beneficial therapeutic effects of special LCFA containing or supplemented with diets[14,15,17-20] are due to at least in part, a similar replacement phenomenon, also remains to be elucidated.

ACKNOWLEDGMENT

Ferenc Pakodi, Áron Vincze, and Ilona Szántó for their help in the technical management of the study.

REFERENCES

1 **Roediger WE.** The colonic epithelium in ulcerative colitis: an energy-deficiency disease? *Lancet* 1980; **2:** 712-715

2 **Cummings JH,** Rombeau JL, Sakata T (eds). Physiological and clinical aspects of short chain fatty acids. Cambridge: *Cambridge University Press,* 1995

3 **Gasbarrini G,** Mingrone G, Giancaterini A, De Gaetano A, Scarfone A, Capristo E, Calvani M, Caso V, Greco AV. Effects of propionyl-L-carnitine topical irrigation in distal ulcerative colitis: a preliminary report. *Hepatogastroenterology* 2003; **50:** 1385-1389

4 **Roediger WE,** Heyworth M, Willoghby P, Piris J, Moore A, Truelove SC. Luminal ions and short fatty acids as markers of functional activity of colon in ulcerative colitis. J Clin Patho Roediger WE, Heyworth M, Willoughby P, Piris J, Moore A, Truelove SC. Luminal ions and short chain fatty acids as markers of functional activity of the mucosa in ulcerative colitis. *J Clin Pathol* 1982; **35:** 323-326

5 **Scheppach W,** Sommer H, Kirchner T, Paganelli GM, Bartram P, Christl S, Richter F, Dusel G, Kasper H. Effect of butyrate enemas oin the colonic mucosa in distal ulcerative colitis.

Gastroenterology 1992; **103:** 51-56
6 **Bieber LL.** Carnitine. *Annu Rev Biochem* 1988; **57:** 261-283
7 **Melegh B,** Kerner J, Bieber LL. Pivampicillin-promoted excretion of pivaloylcarnitine in humans. *Biochem Pharmacol* 1987; **36:** 3405-3409
8 **Costa CC,** de Almeida IT, Jakobs C, Poll-The BT, Duran M. Dynamic changes of plasma acylcarnitine levels induced by fasting and sunflower oil challenge test in children. *Pediatr Res* 1999; **46:** 440-444
9 **Bene J,** Komlósi K, Gasztonyi B, Juhász M, Tulassay Zs, Melegh B. Plasma carnitine ester profile in adult celiac disease patients maintained on long-term gluten free diet. *World J Gastroenterology* 2005; **11:** 6671-6675
10 **Scheppach W,** Muller JG, Boxberger F, Dusel G, Richter F, Bartram HP, Christl SU, Dempfle CE, Kasper H. Histological changes in the colonic mucosa following irrigation with short-chain fatty acids. *Eur J Gastroenterol Hepatol* 1997; **9:** 163-168
11 **Ellestad-Sayed JJ,** Nelson RA, Adson MA, Palmer WM, Soule EH. Pantothenic acid, coenzyme A and human chronic ulcerative and granulomatous colitis. *Am J Clin Nutr* 1976; **29:** 1333-1338
12 **Sandor A,** Kispal G, Melegh B, Alkonyi I. Ester composition of carnitine in the perfusate of liver and in the plasma of donor rats. *Eur J Biochem* 1987; **170:** 443-445
13 **Siguel EN,** Lerman RH. Prevalence of essential fatty acid deficiency in patients with chronic gastrointestinal disorders. *Metabolism* 1996; **45:** 12-23
14 **Esteve-Comas M,** Ramirez M, Fernandez-Banares F, Abad-Lacruz A, Gil A, Cabre E, Gonzalez-Huix F, Moreno J, Humbert P, Guilera M. Plasma polyunsaturated fatty acid pattern in active inflammatory bowel disease. *Gut* 1992; **33:** 1365-1369
15 **Kinsella JE,** Lokesh B, Broughton S, Whelan J. Dietary polyunsaturated fatty acids and eicosanoids: potential effects on the modulation of inflammatory and immune cells: an overview. *Nutrition* 1990; **6:** 24-44; discussion 59-62
16 **Harig JM,** Soergel KH, Komorowski RA, Wood CM. Treatment of diversion colitis with short-chain- fatty acid irrigation. *N Engl J Med 1989;* **320:** 23-28
17 **Stenson WF,** Cort D, Rodgers J, Burakoff R, DeSchryver-Kecskemeti K, Gramlich TL, Beeken W. Dietary supplementation with fish oil in ulcerative colitis. *Ann Intern Med* 1992; **116:** 609-614
18 **Lorenz R,** Weber PC, Szimnau P, Heldwein W, Strasser T, Loeschke K. Supplementation with n-3 fatty acids from fish oil in chronic inflammatory bowel disease - a randomized, placebo-controlled, double-blind cross-over trial. J Intern Med Lorenz R, Weber PC, Szimnau P, Heldwein W, Strasser T, Loeschke K. Supplementation with n-3 fatty acids from fish oil in chronic inflammatory bowel disease--a randomized, placebo-controlled, double-blind cross-over trial. *J Intern Med Suppl* 1989; **731:** 225-232
19 **Loeschke K,** Ueberschaer B, Pietsch A, Gruber E, Ewe K, Wiebecke B, Heldwein W, Lorenz R. n-3 fatty acids only delay early relapse of ulcerative colitis in remission. *Dig Dis Sci* 1996; **41:** 2087-2094
20 **Salomon P,** Kornbluth AA, Janowitz HD. Treatment of ulcerative colitis with fish oil n-3-omega- fatty acid: an open trial. *J Clin Gastroenterol* 1990; **12:** 157-161

S-Editor Kumar M and Guo SY **L- Editor** Elsevier HK **E- Editor** Wang J