Eur J Pediatr (1994) 153:565–568

ORIGINAL PAPER

M. Demirkol
A. C. Sewell
H. Böhles

The variation of carnitine content in human blood cells during disease – a study in bacterial infection and inflammatory bowel disease

Received: 19 January 1993
Accepted: 22 March 1994

M. Demirkol
Department of Paediatrics,
University of Istanbul (Çapa), Turkey

A. C. Sewell · H. Böhles (✉)
Zentrum der Kinderheilkunde,
Theodor Stern Kai 7,
D-60590 Frankfurt/Main, Germany

Abstract Carnitine in erythrocytes and leucocytes represents a small but essential part of the cellular carnitine pool. It was the objective of this study to document the changes of blood cell carnitine concentrations in disease entities with an enhanced cellular metabolism during acute and chronic inflammation. The plasma, erythrocyte, lymphocyte, granulocyte and thrombocyte carnitine concentrations were determined in 23 patients (11.0 ± 8.8 years) with bacterial infections and nine patients (17.5 ± 2.4 years) with Crohn disease and compared to 20 healthy controls (27.0 ± 10.6 years). In patients with bacterial infections the granulocyte carnitine concentrations (126.4 ± 73.5 nmoles/10^6 cells) were higher (P <0.001) than in controls (37.9 ± 22.8 nmoles/10^6 cells). In patients with Crohn disease the lymphocyte carnitine concentrations (169.4 ± 108.2 nmoles/10^6 cells) were increased (P < 0.001) when compared to controls (48.1 ± 18.3 nmoles/10^6 cells). The plasma carnitine concentrations were decreased (P < 0.05) in both patient groups, whereas they were increased (P < 0.05) in the patients' erythrocytes. The carnitine concentrations in thrombocytes did not differ significantly within the individual groups.

Conclusion Lymphocyte and granulocyte carnitine concentrations may reflect their enhanced metabolic state during either immunoglobulin formation or phagocytosis.

Key words Carnitine
Lymphocytes · Granulocytes · Crohn disease · Bacterial infection

Abbreviations *AC* esterified carnitine · *FC* free carnitine *TC* total carnitine

Introduction

Serum carnitine concentrations reflect less than 0.5% of the total body carnitine pool, of which 98% is represented by the muscle mass, the remaining 1.5% being distributed between the different organ systems and blood cells [1, 4, 6]. During inflammatory processes, white blood cells are in an energetically activated state and show an increased fatty acid uptake [9]. As mitochondrial fatty acid metabolism is tightly linked to carnitine, it was the objective of this study to relate the carnitine concentrations of blood cells to defined disease entities with an increased cellular energy requirement such as that during acute and chronic inflammation.

Investigated population

Twenty-three patients aged 11.0 ± 8.8 years with bacterial infections and nine patients aged 17.5 ± 2.4 years with bioptically defined Crohn disease were compared to 20 healthy controls aged 27.0 ± 10.6 years. Patients with bacterial infections were selected on the basis of a positive

Table 1 Distribution of micro-organisms in the group of patients (n = 23) with bacterial infections proved by means of a positive blood culture

Staphylococcus aureus	Osteomyelitis		7
Pneumococci	Pneumonia		5
Haemophilus infl. B			5
	Meningitis	3	
	Sepsis	2	
Meningococcus	Meningitis		3
Mycoplasma	Pneumonia		2
Streptococcus	Pneumonia		1

blood culture (Table 1). They additionally presented with leucocytosis and pathologically increased C-reactive protein and ESR. EDTA-blood (10 ml) was obtained from all patients during the fasting state at about 8 a.m.

Methods and materials

EDTA blood (2 ml) was centrifuged at 1200 × g for 5 min. to obtain plasma.

Isolation of erythrocytes

Red blood cells were washed three times with 0.9% saline and finally disrupted by triple freeze thawing and sonication [2]. The purity of the erythrocytes was controlled by counting the leucocyte nuclei in the preparation. Only a contamination of less than 500 leucocytes/10^8 erythrocytes was accepted.

Isolation of platelets

Platelets were isolated according to Nakagawa [16]. The blood sample was centrifuged for 10 min. at 310 rpm. The obtained platelet rich plasma was transferred to siliconised tubes and centrifuged for another 30 min at 100 g. Platelets were washed once in 10 ml phosphate buffered saline and the obtained cell pellet resuspended in 1 ml distilled water and sonicated for 2 min.

Isolation of lymphocytes and granulocytes

Lymphocytes and granulocytes were prepared according to Boyum [5]. After removal of the platelet rich supernatant, the remaining blood was gently mixed with an equal volume of 0.9% saline. Four millilitres of the diluted blood was carefully layered onto 3 ml Ficol-sodium metrizoate solution (Lymphoprep) and centrifuged for 35 min at 400 g. The upper layer was discarded and the lymphocyte rich suspension at the interface was transferred to a test tube. Six millilitres of Tris-balanced salt solution (Tris-BSS) was added to the lymphocytes and gently resuspended.

Cells were washed 3 times, recentrifuged at 160 g for 10 min, the supernatant discarded and the pellet finally resuspended in 1 ml water and disrupted by triple freeze thawing and sonication.

After removal of platelets and lymphocytes, the remaining blood, containing erythrocytes and granulocytes, was centrifuged and the granulocyte rich supernatant was washed three times. The purity of the lymphocyte and granulocyte preparations was checked by counting the percentage of cells on a smear of the preparation. The overall purity of the used preparations was 93.4% ± 5,3%. Cells were disrupted by triple freeze thawing and additional sonication. The sonicated cell preparations were maintained for 2 weeks at –80°C prior to analysis.

Carnitine determination

The number of cells in the different preparations was counted before lysis using a Coulter counter. Carnitine was determined according to McGarry and Foster [15] with the following modifications: N-(-2-Hydroxyethyl)-Piperazine-N-Ethane Sulphonic acid (HEPES) was used as buffer. Na-tetrathionate was used to bind the free SH-groups of coenzyme A and activated charcoal was used instead of an anion-exchange resin to bind the surplus ^{14}C-acetyl-CoA. Concentrations were expressed per 10^8 erythrocytes and thrombocytes and per 10^6 granulocytes and lymphocytes. In our hands the interassay coefficient of variation of carnitine determination was: total carnitine 1.5%; free carnitine 2.6%; short chain acyl carnitine: 12.0%. The corresponding intra-assay coefficient of variation was: total carnitine 1.9%; free carnitine 2.3%; short chain acyl carnitine 10.8%. To exclude a possible quench effect of haemoglobin on the carnitine determination crystallized and dialysed haemoglobin (Sigma, München) was added (10–15 g Hb/dl, corresponding to the amount of haemoglobin in the erythrocyte preparations of our patients) to a 20 μM or 40 μM L-carnitine solution and the resulting counts compared to those of a carnitine solution without haemoglobin. The resulting counts were: 20 μM carnitine without haemoglobin 432 cpm; with haemoglobin 446 cpm; 40 μM carnitine without haemoglobin 823 cpm; with haemoglobin 815 cpm. As the coefficient of variation determined was 2.3% for free carnitine, haemoglobin was considered not to influence the results of our erythrocyte carnitine determinations.

Statistical methods

The mean and standard deviations were calculated with the BIO85 version 1 Biomedical statistical program. Between group comparisons were performed with the Mann-Whitney U-test.

Results

The concentrations of total (TC), free (FC) and esterified carnitine (AC) in plasma and the different blood cells are given in Table 2. When compared to controls, plasma TC and FC concentrations were significantly decreased in patients with either bacterial infection or inflammatory bowel disease ($P < 0.05$). The TC and FC concentrations in erythrocytes were significantly increased ($P < 0.05$) in patients with Crohn disease when compared with either healthy controls or patients with bacterial infections. The latter group did not differ from controls. TC- and AC concentrations in lymphocytes were significantly higher in patients with Crohn disease than in controls ($P < 0.001$ and $P < 0.01$), whereas they did not differ from controls in patients with bacterial infections. In granulocytes of patients with bacterial infections the TC ($P < 0.001$), FC ($P < 0.01$) and AC ($P < 0.001$) concentrations were increased compared to healthy controls. They also tended to be higher when compared to those in Crohn disease, however only the FC concentrations were significantly increased ($P < 0.05$).

Table 2 Plasma and blood cell carnitine concentrations (mean ± 1 SD) in healthy controls (group 1) and patients with bacterial infections (group 2) and patients with Crohn disease (group 3)

Controls (n = 20)	Plasma (ml)	Erythrocytes (10^8 cells)	Lymphocytes (10^6 cells)	Granulocytes (10^6 cells)	Thrombocytes (10^8 cells)
Total Carnitine (nmoles/–)	31.6 ± 7.7*[1, *4]	22.9 ± 10.8	48.1 ± 18.3	37.9 ± 22.8	31.1 ± 24.0
Free Carnitine (nmoles/–)	19.9 ± 5.7*[1, *4]	6.3 ± 5.8	15.3 ± 15.2	11.6 ± 11.2	11.1 ± 6.5
Acylcarnitine (nmoles/–)	11.2 ± 3.6	16.3 ± 9.5	34.3 ± 31.5	31.6 ± 21.7	21.2 ± 23.2
AC/FC	0.56	2.59	2.24	2.72	1.91
% Acylation	35.4	71.2	71.3	83.4	68.2
Bacterial infection (n = 23)					
Total Carnitine (nmoles/–)	26.7 ± 9.3	29.2 ± 18.4	83.6 ± 74.5	126.4 ± 73.5*[3]	45.4 ± 44.0
Free Carnitine (nmoles/–)	15.3 ± 5.9	5.7 ± 4.4	34.4 ± 41.2	34.4 ± 38.0*[2, *7]	14.3 ± 30.9
Acylcarnitine (nmoles/–)	11.8 ± 8.2	23.0 ± 16.5	51.6 ± 53.6	91.9 ± 72.2*[3]	29.7 ± 40.9
AC/FC	0.77	4.04	1.50	2.67	2.08
% Acylation	44.2	78.8	61.7	72.7	65.4
Crohn Disease (n = 9)					
Total Carnitine (nmoles/–)	22.9 ± 8.8	36.6 ± 20.3*[4, *7]	169.4 ± 108.2*[6, *7]	73.9 ± 62.5	45.1 ± 34.5
Free Carnitine (nmoles/–)	14.4 ± 5.6	15.0 ± 14.4*[4, *7]	46.9 ± 48.3	11.6 ± 15.4	6.9 ± 7.2
Acylcarnitine (nmoles/–)	8.5 ± 6.6	21.6 ± 13.5	122.5 ± 96.4*[5, *7]	62.3 ± 53.4	38.1 ± 33.6
AC/FC	0.59	1.44	2.61	5.37	5.52
% Acylation	37.1	59.0	72.3	84.3	84.5

*[1] Group 1 vs. 2 ($P < 0.05$)
*[2] Group 1 vs. 2 ($P < 0.01$)
*[3] Group 1 vs. 2 ($P < 0.001$)
*[4] Group 1 vs. 3 ($P < 0.05$)
*[5] Group 1 vs. 3 ($P < 0.01$)
*[6] Group 1 vs. 3 ($P < 0.001$)
*[7] Group 2 vs. 3 ($P < 0.05$)

The carnitine concentrations in thrombocytes did not differ significantly within the individual groups.

Discussion

The present study confirms previous data showing that blood cells contain significant amounts of carnitine [1, 4, 7, 13]. This is surprising in the case of erythrocytes, because they lack mitochondria and are entirely energetically independent of fatty acid oxidation. Therefore there is no demand for a classical carnitine mediated fatty acid transport in these cells. Remarkably, the percentage of AC is significantly higher in erythrocytes than in plasma, which is confirmed by others [6, 12]. Nevertheless there is some controversy about red cell carnitine concentrations with one group actually denying any presence of carnitine in erythrocytes [13]. In the meantime however, there is no doubt about the presence of significant amounts of carnitine in erythrocytes which is recently presumed to exert a stabilizing effect on the erythrocyte membrane by palmitoyltransferase catalysed acyl transfer from acylcarnitine to phospholipids [2, 3].

It is unanimously accepted that mononuclear as well as polymorphonuclear cells contain appreciable amounts of carnitine [1, 7, 8, 14]. However, the range of determined carnitine concentrations is wide and very much under debate. Our results (granulocytes: 37.9 ± 22.8 µmoles TC/10^9 cells; lymphocytes: 48.1 ± 18.3 µmoles TC/10^9 cells) confirm the leucocyte carnitine concentrations measured by Katrib et al. [13, 14] (granulocytes: 52.2 ± 16.9 µmoles TC/10^9 cells; lymphocytes: 41.9 ± 9,3 µmoles/10^9 cells). However these concentrations were critizised by Fürst and Glöggler [11] as too high and it was speculated that they may be caused by the use of TRIS buffer. As we used HEPES buffer in our carnitine assay, resulting in leucocyte carnitine concentrations similar to those of Katrib et al. [13] we can disprove this speculation.

The increased carnitine concentrations in granulocytes and lymphocytes were clearly related to specific disease entities. Granulocytes are in the first line of defence during acute bacterial infections. The increased granulocyte carnitine concentrations may therefore reflect the stimulated energetic state of these phagocytic cells during acute inflammation [1], using mitochondrial fatty acid oxidation as their energy source. The carnitine concentrations of lymphocytes, the cells mediating specific immunity, were significantly increased in patients with Crohn disease, representing chronic inflammation. The peripheral and intestinal lymphocytes of patients with Crohn disease manifest increased immune responses to cross-reacting bacterial and colonic antigens. Therefore this increase is equally interpreted as reflecting their enhanced cellular energy metabolism. This idea is supported by the results of an in vitro study showing that phytohaemagglutin-induced peripheral blood lymphocyte proliferation was

markedly increased in lymphocytes preloaded with carnitine [10].

Our results demonstrate that the carnitine concentration of peripheral blood cells is obviously related to their actual metabolic activity. In defined disease entities the carnitine content of individual cell populations is selectively stressed and carnitine may become limiting for the required cell reactivity during disease control.

References

1. Adlouni HA, Katrib K, Ferard G (1988) Changes in carnitine in polymorphonuclear leukocytes, mononuclear cells and plasma from patients with inflammatory disorders. Clin Chem 34: 40–43
2. Arduini A, Tyurin V, Tyuruna Y, Arrigoni-Martelli E, Molajoni F, Dottori S, Federici G (1992) Acyl-trafficking in membrane phospholipid fatty acid turnover: the transfer of fatty acid from the acyl-L-carnitine pool to membrane phospholipids in intact human erythrocytes. Biochem Biophys Res Commun 187: 353–358
3. Arduini A, Mancinelli G, Radatti GL, Dottori S, Molajoni F, Ramsay RR (1992) Role of carnitine and carnitine palmitoyltransferase as integral components of the pathway for membrane phospholipid fatty acid turnover in intact human erythrocytes. J Biol Chem 267: 12673–12681
4. Borum PR, York CM, Bennett SG (1985) Carnitine concentrations of red blood cells. Am J Clin Nutr 41: 653–656
5. Boyum A (1968) Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest 21 [Suppl 97]: 77–89
6. Cooper MB, Forte CA, Jones DA (1988) Carnitine and acylcarnitine in red blood cells. Biochim Biophys Acta 959: 100–105
7. Cress AP, Fraker PJ, Bieber LL (1989) Carnitine and acylcarnitine levels of human peripheral blood lymphocytes and mononuclear phagocytes. Biochim Biophys Act 992: 135–139
8. Deufel T (1990) Determination of L-Carnitine in biological fluids and tissues. J Clin Chem Biochem 28: 307–311
9. Elsbach P (1959) Composition and synthesis of lipids in resting and phagocyting leucocytes. J Exp Med 110: 969–980
10. Franceschi C, Cossarizza A, Troiano L, Salati R, Monti D (1990) Immunological parameters in aging: Studies on natural immunomodulatory and immunoprotective substances. Int J Clin Pharmacol Res 10: 53–57
11. Fürst P, Glöggler A (1987) Reappraisal of carnitine concentrations in blood. Clin Chem 22: 1956–1957
12. Harper P, Wadstrom C, Cederblad G (1993) Carnitine measurements in liver, muscle tissue and blood in normal subjects. Clin Chem 39: 592–599
13. Katrib K, Adlouni HA, Ferard G (1987) Presence of nonesterified and acylcarnitine in human polymorphonuclear leukocytes and mononuclear cells. Clin Chem 33: 533–535
14. Katrib K, Adlouni AH, Férard G (1987) Carnitine in human polymorphonuclear leucocytes, mononuclear cells and platelets. Am J Clin Nutr 46: P734–735
15. McGarry JD, Foster DW (1976) An improved and simplified radioisotopic assay for the determination of free and esterified carnitine. J Lipid Res 17: 277–281
16. Nakagawa Y (1978) Isolation of platelets from human blood cells. J Lab Clin Med 91: 922–9289