

Serum carnitine and acyl-carnitine in patients with meningitis due to tick-borne encephalitis virus infection

Alina Kępką^{1, A–D, F}, Roman M. Janas^{1, C, E, F}, Sławomir A. Pancewicz^{2, A, C, F}, Renata Świerżbińska^{2, B, F}

¹ Department of Biochemistry, Radioimmunology and Experimental Medicine, the Children's Memorial Health Institute, Warszawa, Poland

² Department of Infectious Diseases and Neuroinfections, Medical University of Białystok, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

Advances in Clinical and Experimental Medicine, ISSN 1899-5276 (print), ISSN 2451-2680 (online)

Adv Clin Exp Med. 2017;26(2):277–280

Address for correspondence

Alina Kępką
E-mail: a.kepka@czd.pl

Funding sources

none declared

Conflict of interest

none declared

Acknowledgements

Instruments used in this study were purchased as a part of project No. POIG.02.01.00-14-059/09-00 co-financed by the European Union under the European Regional Development Fund.

Received on December 22, 2015

Revised on April 5, 2016

Accepted on May 4, 2016

Abstract

Background. Hard ticks are the main vectors of tick-borne encephalitis virus (TBEV). Free carnitine (FC) and acylcarnitines (AC) have the basic role in β -oxidation as well as the modulation of immune and nervous system. Homeostasis of carnitines in the TBE patients was not studied so far.

Objectives. This study aimed to evaluate FC and AC serum concentrations in patients with meningitis due to TBEV infection before and after 14 ± 3 days of treatment.

Material and methods. The study was performed in 14 patients aged 48 ± 29 years that were divided a posteriori (based on their FC level before and after treatment) into 2 subgroups: 1–8 and 9–14. Diagnosis was based on the neurological, serological and pleocytosis evaluation.

Results. The FC level in patients 1–8 before treatment (24.1 ± 8.1) was significantly lower than in patients post-treatment (34.4 ± 8.3), lower than in the control group (40.5 ± 7.6), and lower than in patients 9–14 before treatment (40.0 ± 13.5) but not lower than in the patients 9–14 after treatment (24.7 ± 7.3 $\mu\text{mol/L}$), respectively, $p < 0.05$. AC concentration in the patients 1–8 before treatment (4.7 ± 2.2) was apparently lower than in patients post-treatment (9.5 ± 3.9 $\mu\text{mol/L}$) but the values were not significantly different. In patients 9–14 before treatment the AC concentration (16.3 ± 12.6) was higher than in patients after treatment (5.3 ± 4.0 $\mu\text{mol/L}$), but the difference was not statistically significant.

Conclusions. FC and AC homeostasis in circulation was disturbed in the patients with meningitis due to TBEV infection patients. The mean levels of FC and AC in 60% of the patients were below the normal range but normalized after treatment whereas in 40% of the patients they were near or at a normal range and significantly decreased after treatment. Explanation of this intriguing finding and its clinical significance is not easy without further studies.

Key words: carnitine deficiency, tick-borne encephalitis, free carnitine, acyl-carnitine

DOI

10.17219/acem/63006

Copyright

Copyright by Author(s)

This is an article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Introduction

Tick-borne encephalitis (TBE) RNA virus (TBEV) from the *Flavivirus* genus belongs to the neurotropic viruses. Infection with the TBEV is initiated by a bite from an infected hard tick (i.e. *Ixodes ricinus*). The virus is present in the tick's saliva where there is a natural anesthetic; therefore, the tick bite may be unnoticed. The ticks may transmit more than one pathogen, thus complicating diagnosis and treatment of the TBE.¹ In 65–70% of TBEV infected humans, the virus does not cause any symptoms but in symptomatic patients the disease develops in 2 stages. First signs may include high temperature, headache, tiredness, muscle pain, which last 1–8 days after which most of the patients recover. In the second phase, the brain and spinal cord become affected by the virus that manifests in: meningitis, meningoencephalitis or meningoencephalomyelitis.^{2,3}

Free carnitine (FC) and acylcarnitines (AC) are important, multifactorial substances. The majority of the FC originates from the diet, whereas about 25% is synthesized de novo from lysine and methionine in the liver, kidney and brain but not in the skeletal and cardiac muscles. FC provides fatty acids as well as the products of their peroxisomal partial oxidation into the mitochondria for the β -oxidation.^{4,5} It removes an excess of toxic acyl-CoA from mitochondrial matrix, regulates acyl-CoA/free CoA ratio, and supplies acetyl groups for the synthesis of acetylcholine and for acetylation of nuclear histones in the central nervous system.⁶ "Secondary" roles of carnitine include its actions as an immune system, gene and protein modulator, antioxidant, anti-inflammatory and anti-apoptotic factor.⁷ Low serum carnitine levels were shown in patients with impaired immune reactions, metabolic disorders, recurrent infections, and chronic fatigue syndrome.^{4,5,8} Carnitine deficiency may result from

the malnutrition, malabsorption, peritoneal dialysis, and increased urinary AC excretion.^{5,9} FC and AC may have an unexplored role in the pathophysiology of the tick-borne encephalitis resulting from TBE virus infection. Therefore, we aimed to evaluate serum concentration of the FC and AC in adult patients with meningitis resulting from TBEV infection, before and after supportive and symptomatic treatment.⁸ Their homeostasis in the TBE patients was not studied so far.

Material and methods

Patients

The study group included 14 adults (6 male, 8 female) aged 48 ± 29 years with meningitis resulting from TBEV infection. Diagnosis was based on the neurological evaluation and serological determinations in the sera and cerebrospinal fluid (CSF). Upon admission all the patients exhibited typical symptoms seen in the TBE virus infection: fever lasting 1–3 weeks, headache, arthralgia, myalgia. Supportive and symptomatic treatment of the patients lasted 14 ± 3 days. The study group was divided a posteriori (based on the results of the FC determinations before and after treatment) into 2 subgroups: patients number 1–8 (4 male, 4 female) aged 44 ± 18 years, in whom the FC concentration increased as a result of treatment and patients number 9–14 (2 male, 4 female) aged 51 ± 19 years, in whom the FC concentration decreased after treatment. Serological and biochemical characteristics of both subgroups are shown in Table 1. Exclusion criteria: primary carnitine deficiency, patients with borreliosis, other recent viral infections, genetic and metabolic disorders, heart, renal and hepatic failure. The control group consisted of 32 healthy male ($n = 17$) and female ($n = 15$) aged 42 ± 11 years.

Table 1. Cerebrospinal fluid (CSF) and serum parameters in the TBE patients exhibiting increase (subgroup 1–8) or decrease (subgroup 9–14) of free carnitine (FC) concentration after 14 ± 3 days of treatment

Biological fluid	Parameters	Subgroup 1–8		Subgroup 9–14		Reference values
		before treatment	after treatment	before treatment	after treatment	
mean \pm SD						
CSF	total protein	67.6 \pm 12.9	66.7 \pm 45.9	56.7 \pm 18.2	44.2 \pm 3.4	15–45 mg/dL
	albumin	43.0 \pm 9.8	24.4 \pm 10.9	41.3 \pm 12.8	nd	10–30 mg/dL
	glucose	56.8 \pm 5.9	60.4 \pm 11.3	62.0 \pm 7.7	49.5 \pm 0.5	48–85 mg/dL
	chloride	119 \pm 2.6	118 \pm 1.4	113 \pm 1.1	117 \pm 1.5	120–130 mM/L
	pleocytosis	112 \pm 52.4	34.8 \pm 19.8	58.8 \pm 28.8	22.0 \pm 2.0	< 5 cells/ μ L
median (range)						
	IgM	30.2 (2.1, 147)	nd	1.9 (1.8, 351)	nd	antibody index < 1.4
	IgG	29.8 (6.0, 75.0)	nd	11.6 (0.0, 87.0)	nd	cut-off < 10 U/mL
Serum	IgM	45.7 (6.5, 207)	nd	9.3 (8.1, 500)	nd	antibody index < 1.4
	IgG	56.3 (19.1, 156)	nd	79.2 (22.0, 115)	nd	cut-off < 10 U/mL

Data are mean \pm SD or median and range; nd – not determined.

Methods

Serological determinations

IgM and IgG antibodies against TBEV in the serum and CSF samples were assayed using ELISA Enzygnost Anti-TBE/FSME Virus [IgG, IgM] kit from Siemens, Marburg, Germany. Performance of the assay and interpretation of the data was done according to the Manufacturer's instruction. The data consistently indicated recent TBEV infection in our patients.

Carnitine assay

Free (FC) and total (TC) carnitine was determined in duplicates as previously detailed.^{9,10} FC was measured in the serum filtrates without hydrolysis of acyl-carnitines, based on the reaction of FC with acetyl-CoA and carnitine acetyltransferase. TC concentration was quantified in the serum filtrates after hydrolysis of the carnitine esters: TC – FC = AC. Sensitivity of the method was 4.0 µmol/L. Inter- and intra-assay coefficient of variation (CV%) was 4.0 and 7.0%, respectively.

Statistical analysis

Data is expressed as mean ± SD or median (range) and was analyzed with STATISTICA (v. 8.0, StatSoft, Poland), using the Student t-test for independent samples. The normality of the variables distribution was tested using Shapiro-Wilk statistics and normality plots. Differences between 2 independent groups were analyzed using U Mann-Whitney non-parametric test. Differences between patients before and after treatment were tested using the Wilcoxon signed rank test. Statistical significance was assumed at $p < 0.05$.

Results

Serum concentrations of FC measured before and after 14 days of treatment in 14 patients with meningitis resulting from TBE virus infection are shown in Fig. 1. In the patients 1–8 the FC levels increased, whereas in the patients 9–14 they decreased after treatment. Before treatment, FC levels in patients 1–8 and 10, 13 were below normal range, whereas in patients 9, 11, 12, 14 within the normal range.

Mean concentrations of the FC and AC are shown in Table 2. Mean FC levels in the patients 1–8 before treatment (24.1 ± 8.1 µmol/L) were significantly lower ($p < 0.05$) than in patients post-treatment (34.4 ± 8.3 µmol/L), and lower than in healthy controls (40.5 ± 7.6 µmol/L), lower than in patients 9–14 before treatment (40.0 ± 13.5 µmol/L), but not lower than in patients 9–14 post-treatment (24.7 ± 7.3 µmol/L), $p > 0.05$. As a result of treatment,

Table 2. Mean serum free carnitine (FC) and acylcarnitine (AC) concentration in the patients from subgroups 1–8 and 9–14 with meningitis due to tick-borne encephalitis virus (TBEV) infection before and after treatment

Group	Patients	FC (µmol/L)	AC (µmol/L)
(I)	control group	40.5 ± 7.6	13.5 ± 8.4
TBE patients			
(II)	1–8 (before)	24.1 ± 8.1	4.7 ± 2.2
(III)	1–8 (after)	34.4 ± 8.3	9.5 ± 3.9
(IV)	9–14 (before)	40.9 ± 13.5	16.3 ± 12.5
(V)	9–14 (after)	24.7 ± 3.7	5.3 ± 4.0
p-values			
	II vs I	< 0.000	< 0.007
	III vs I	ns	ns
	IV vs I	ns	ns
	V vs I	< 0.000	< 0.001
	II vs III	< 0.011	ns
	IV vs V	< 0.027	ns
	II vs IV	< 0.016	ns
	II vs V	ns	ns
	III vs IV	ns	ns
	III vs V	< 0.045	< 0.033

values are mean ± SD; ns – not significant; vs – versus.

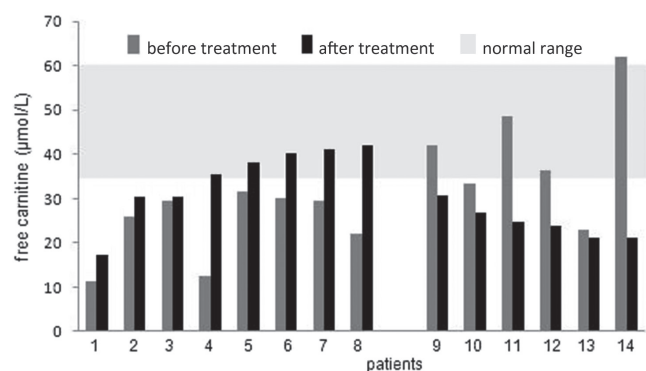


Fig. 1. Serum free carnitine in patients with meningitis due to tick-borne encephalitis virus infection before and after 14 days of treatment

FC level in the patients 1–8 reached the values seen in healthy controls (Table 2). In contrast, FC level in patients 9–14 before treatment was the same (40.9 ± 13.5 µmol/L) like in the control group (40.5 ± 7.6 µmol/L) and statistically significantly decreased (24.7 ± 3.7 µmol/L) after treatment ($p < 0.05$) (Table 2).

Mean AC concentration in the patients 1–8 before treatment (4.7 ± 2.2 µmol/L) was apparently lower than that after treatment (9.5 ± 3.9 µmol/L), but the difference did not reach statistical significance, although it was significantly lower than that seen in healthy controls (13.5 ± 8.4 µmol/L, $p < 0.001$) (Table 2). In the patients 9–14 before treatment the AC concentration (16.3 ± 12.5 µmol/L) was apparently higher than that seen

after treatment ($5.3 \pm 4.0 \mu\text{mol/L}$), but the difference was not statistically significant ($p > 0.05$), Table 2.

In the patients 1–8, IgM index (positive > 1.4) and IgG level (cut-off 10 standard units, U/mL) at admission was (median and range) 45.7 (6.5, 207) and 56.3 (19.1, 156), and in the CSF 30.2 (2.1, 147) and 29.8 (6.0, 75.0), respectively. In the patients 9–14 IgM index and IgG concentration (U/mL) was 9.3 (8.1, 500) and 79.2 (22.0, 115), and in the CSF 1.9 (1.8, 351) and 11.6 (0.0, 87.0), respectively. Pleocytosis (cut-off $< 5 \text{ cells}/\mu\text{L}$) in the patients 1–8 at admission was $112 \pm 52.4 \text{ cells}/\mu\text{L}$ and after treatment: $34.8 \pm 19.8 \text{ cells}/\mu\text{L}$, whereas in the patients 9–14 it was $58.8 \pm 28.8 \text{ cells}/\mu\text{L}$ and $22.0 \pm 2.0 \text{ cells}/\mu\text{L}$, respectively. There were no statistically significant differences between biochemical parameters, IgG and IgM titers and pleocytosis between subgroups as well as between subgroups before and after treatment, respectively (Table 1).

Discussion

In this preliminary, observational study we have shown that FC and AC homeostasis in circulation is disturbed in the patients with meningitis due to TBEV infection. Supportive and symptomatic treatment of the patients and/or their natural healing process had an impact on the carnitine levels. Upon admission, the majority of our patients exhibited a substantial deficiency of the serum FC. Energy-dependent immunological mobilization of the infected organism (synthesis of the IgG/IgM anti-TBEV antibodies, anti- and pro-inflammatory cytokines) and decreased food intake may be responsible for the lowering of carnitines in circulation and tissues.^{4,5} Normal availability of FC is crucial for the efficient β -oxidation, generation of AC and supply of the energy. Although glucose, not fatty acids, is a major source of energy in the brain, it has been shown that FC and AC deficiency (i.e. due to starvation) may cause metabolic encephalopathy.⁸ About 99% of the carnitines are present inside the cells, but it is believed that circulating fraction of FC, TC and AC reflects their homeostasis in whole organism.⁵

Although all the patients were recovering well from the disease, we have noticed 2 types of patients. FC and AC serum levels in patients 1–8 prior to treatment were be-

low the normal range, and significantly increased as a result of treatment. In contrast, FC and AC levels in patients 9–14 prior to treatment were significantly higher than those in the patients 1–8 before treatment and decreased below the normal range after treatment (Fig. 1, Table 2). Explanation of this intriguing finding is not easy without further basic and clinical studies on a numerous groups of patients.

Due to the small number of patients, it was not possible (or allowed) to perform an analysis of the correlations between carnitine concentrations and biochemical parameters, pleocytosis and IgM and IgG titers. It is tempting to speculate that differences in metabolic rate and immune system among the patients may be responsible for the observed phenomenon.

Some symptoms that accompany various clinical types of the TBE may co-result from the deficiency of carnitines. Carnitines supplementation has been shown to improve the overall energy status of the brain and the whole organism and to ameliorate symptoms of various diseases including neurological disturbances.⁷ However, this topic also requires further studies.

References

1. Lani R, Moghaddam E, Haghani A, Chang LY, AbuBakar S, Zandi K. Tick-borne viruses: A review from the perspective of therapeutic approaches. *Ticks Tick Borne Dis.* 2014;5:457–465.
2. Holzmann H. Diagnosis of tick-borne encephalitis. *Vaccine.* 2003; 21 Suppl 1:36–40.
3. Lindquist L, Vapalahti O. Tick-borne encephalitis. *Lancet.* 2008;371: 1861–1871.
4. Famularo G, De Simone C, Trinchieri V, Mosca L. Carnitines and its congeners: A metabolic pathway to the regulation of immune response and inflammation. *Ann N Y Acad Sci.* 2004;1033:132–138.
5. Flanagan JL, Simmons PA, Vehige J, Willcox MD, Garrett Q. Role of carnitine in disease. *Nutr Metab (Lond).* 2010;7:30–34.
6. Madiraju P, Pande SV, Prentki M, Madiraju SR. Mitochondrial acetyl-carnitine provides acetyl groups for nuclear histone acetylation. *Epigenetics.* 2009;4:399–403.
7. Ribas GS, Vargas CR, Wajner M. L-carnitine supplementation as a potential antioxidant therapy for inherited neurometabolic disorders. *Gene.* 2014;533:469–476.
8. Jones LL, McDonald DA, Borum PR. Acylcarnitines: Role in brain. *Prog Lipid Res.* 2010; 49:61–75.
9. Kępka A, Minarowska A, Minarowski Ł, et al. Serum and urinary carnitine in children with cystic fibrosis. *Prog Health Sci.* 2013;3:13–18.
10. Cederblad G, Harper P, Lindgren K. Spectrophotometry of carnitine in biological fluids and tissue with a Cobas Bio centrifugal analyzer. *Clin Chem.* 1986;322:342–346.