Dig Dis Sci (2008) 53:1114–1121
DOI 10.1007/s10620-007-9983-1

ORIGINAL PAPER

L-Carnitine Treatment Reduces Steatosis in Patients with Chronic Hepatitis C Treated with α-Interferon and Ribavirin

Marcello Romano · Marco Vacante · Erika Cristaldi · Valentina Colonna · Maria Pia Gargante · Lisa Cammalleri · Mariano Malaguarnera

Received: 7 February 2007 / Accepted: 15 August 2007 / Published online: 16 October 2007

Abstract *Background* Hepatic steatosis is a common presentation in patients with chronic hepatitis C. Interferon α exerts both antiviral and immunomodulating actions, and influences on lipid metabolism. The aim of our study was to test whether L-carnitine reduces steatosis in patients treated with interferon and ribavirin. *Patients and methods* A total of 70 patients were randomly assigned to receive either leucocyte IFN alpha at a dose of 3 MIU thrice a week plus 1,000 mg ribavirin per day for 12 months (group A) or IFN alpha and ribavirin at the same dose plus 2 g carnitine per day (group B). *Results* Comparison of the two treatments showed significant differences between the mean values of the following parameters at the end of the treatment: ALT –68 vs –95 IU/ml ($P < 0.05$), total cholesterol 0.08 vs –0.91 mmol/l ($P < 0.05$) and triglycerides +0.25 vs –20 mmol/l ($P < 0.05$); and at the follow-up: AST –35 vs –65 IU/ml ($P < 0.05$) and ALT –55 vs –84 IU/ml ($P < 0.05$). All values were lower in group B (IFN + Ribavirin + Carnitine) than in group A (IFN plus Ribavirin). When comparing those patients treated with IFN + ribavirin with those treated with IFN plus ribavirin plus carnitine, the response at the end of the treatment was 48% vs 56%, and the sustained response 39% vs 46%, respectively. *Conclusions* Combined treatment with L-carnitine, ribavirin and IFN alpha resulted in greater antihyperlipidaemic effects and than with ribavirin and IFN alpha alone. The results of this study suggest that L-carnitine may have a role among the reduction of steatosis strategies in patients with hepatitis C treated with IFN alpha and ribavirin.

Keywords Hepatic steatosis · Antihyperlipidaemic effects · HCV · Interferon alpha · Ribavirin

M. Romano · M. Vacante · E. Cristaldi · V. Colonna · M. P. Gargante · L. Cammalleri · M. Malaguarnera (✉)
Department Of Senescence, Urological And Neurological Sciences Ospedale Cannizzaro, Viale Messina 829-95125 Catania, Italy
e-mail: malaguar@unict.it

Introduction

Hepatitis C virus (HCV) is one of the major of chronic hepatitis types and represents an important risk factor for steatosis, cirrhosis and hepatocellular carcinoma. During the last 4 years, several clinical trials have suggested that liver steatosis might play an important role in the outcome of treated hepatitis C.

Previous studies carried out in vivo and in vitro showed that HCV core protein might induce steatosis in transfected cells [1] and transgenic mice [2]. Genotype 3a seems to induce steatosis with no accompanying risk factors; whereas, obesity is often observed with steatosis associated with genotype 1b [3]. Liver steatosis occurs in approximately 50% of these patients [4, 5]. Steatosis of the liver is a frequent bioptic finding in patients infected with HCV, and is extensive and severe in some. It has been demonstrated that steatosis associated with being overweight could be a predictive factor for liver fibrosis in patients infected with HCV [6, 7].

Treatment with interferon alpha (IFNα) in combination with ribavirin is currently the therapeutic option with proven efficacy [8, 9]. This drug not only exerts antiviral and immunomodulating actions, but also seems to influence lipid metabolism [10].

IFNα induces a reduction of serum high density lipoprotein cholesterol (HDL-C) levels, as well as increasing triglyceride levels and, consequently, the amount of fat in

the liver [11, 12]. In order to reduce this risk condition for the development of liver steatosis, we added carnitine administration to the usual IFNα and ribavirin treatment. In fact, carnitine is a water-soluble polarised substance that plays a fundamental role in the production and dissemination of cellular energy and is involved in numerous metabolic steps. Among these, the most important seem to be: the utilisation of substrates for cellular energy production in the mitochondria; lipid oxidation inside peroxisomes, unrelated to energy production; acylation and deacylation of proteins such as very low-density lipoprotein (VLDL) at the reticular endoplasmic level; regulation of cell surface phospholipid turnover and maintenance of cellular osmotic balance [13]. On the basis of previously reported data, we compared the effects of the IFNα and ribavirin combined with oral carnitine administration versus IFNα and ribavirin treatment alone on liver steatosis in patients with chronic active hepatitis C [14–17].

Patients and methods

Patients

From June 2000 to December 2003 we evaluated all consecutive adult patients with chronic hepatitis admitted to our department.

About 70 patients with chronic hepatitis C diagnosed by clinical, humoral and histological findings were eligible for this randomised study. Inclusion criteria were: elevated serum levels of alanine aminotransferase (ALT) at least twice the normal values for more than 12 months and the presence of anti-HCV antibodies in the serum, the HCV-RNA being more than 1,000 copies/ml. Exclusion criteria included: positive test for serum hepatitis B surface antigen, positive test for serum HIV antibodies, alcohol-induced liver disease (daily alcohol consumptions greater than 50 g), liver cirrhosis or malnutrition, infection, previous organ transplantation, other causes of liver disease, pre-existing psychiatric disease, seizure disorders, cardiovascular disease, haemoglobinopathies, poorly controlled diabetes or autoimmune-type disease, or any concomitant medical illness requiring treatments of corticosteroids, β-blockers, diuretics or any drug that might influence serum lipid levels. Gender, age, age at infection, body mass index, presumed mode of infection (parenteral, usually intravenous drug use, transfusion, other or unknown) were assessed (Table 1).

Informed consent was obtained from each patient in agreement with the ethics guidelines of the Declaration of Helsinki, 1975, as reflected in a priori approval by the Institution's human research Committee.

Methods

The patients who met the inclusion criteria for enrolment in the study were randomly assigned to two groups (A or B) on the basis of a computer-generated randomisation schedule. Eligible patients were randomly assigned to a two-treatment study, in equal proportion, stratified by HCV genotype (1b versus others) and viraemia.

Group A received leucocyte IFNα (Alfaferone; Alfa Wasserman Italy) intramuscularly at a dosage of 3 MIU thrice-a-week for 12 months, plus ribavirin (1,000 mg). Group B received the same as group A with the additional treatment of carnitine (2 g *per os* once-a-day).

The dosage of ribavirin was reduced to 600 mg/daily and then to 400 mg daily, and haemoglobin levels decreased to less than 12 g/dl; treatment was discontinued if the level decreased to less than 8.5 g/dl.

Blood was drawn after an overnight fast and the samples were frozen at –80°C within 2 h. Patients were followed up for a 6-month period after the end of the planned treatment [18].

Clinical and laboratory evaluation

All participants gave a full clinical history and underwent a physical examination. Laboratory studies were conducted at baseline, weekly for the first 6 weeks, at week 8 and then every 4 weeks during the treatment phase. In addition tests were performed at 12 and 24 weeks after completion of therapy.

The tests included haemoglobin, white cell count, platelets–prothrombin time, blood urea nitrogen, serum creatinine, bilirubin, ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and y-glutamil transpeptidase levels. Haemoglobin level was checked more frequently if it decreased by more than 2 g/dl or to less than 10 g/dl.

The total cholesterol, HDL-C, LDL-C and triglycerides were also evaluated at baseline, at 3, 6, 9, 12 months and at follow-up. An enzymatic method was used to determine serum total cholesterol and triglyceride levels (Hitachi 704 analyser—twin TG/CHO reactive, Boehringer Mannheim automated analysis, Germany). HDL-C was determined using another enzymatic method after precipitation of lipoproteins containing apolipoprotein B with phosphotungstic acid/magnesium chloride. LDL-C levels were computed using Friedewald's method.

Virological findings

Anti-HCV antibodies were evaluated using second generation enzyme-linked immunosorbent assay ELISA (Ortho-Diagnostic Systems, Raritan NJ, USA), and positive samples have been confirmed by means of immunoblotting

Table 1 Patients' characteristics at the time of liver biopsy

Parameter	Normal value	Group A (IFNα and ribavirin)	Group B (IFNα and ribavirin + carnitine)	*P* value
No		35	35	n.s.
Age (years)		50.4 ± 5.6	50.1 ± 6.1 n.s.	n.s.
Gender (male/female)		16/14	17/13 n.s.	n.s.
Time since exposure (years)		6.08 ± 3.2	6.12 ± 3.0	n.s.
Body mass index		25.7 ± 3.2	25.8 ± 3.1	n.s.
Probable exposure (no. of patients)				
Blood transfusion		16	15	n.s.
Infected needle		6	7	n.s.
Healthcare environment		4	4	n.s.
Other/unknown		9	9	n.s.
Laboratory parameter				
Aspartate aminotransferase (IU/l)	20–50	155 ± 32	158 ± 31	n.s.
Alanine aminotransferase (IU/l)	20–50	170 ± 44	175 ± 42	n.s.
Alkaline phosphatase (IU/l)	38–126	187 ± 51	189 ± 47	n.s.
Prothrombin time (%)	70–110	93 ± 8	94 ± 11	n.s.
γ-Glutamyltransferase (IU/l)	8–78	125 ± 38	130 ± 24	n.s.
Cholesterol (mmol/l)	3.89–6.48	5.08 ± 0.76	5.09 ± 0.81	n.s.
Triglycerides (mmol/l)	0.79–1.92	2.01 ± 0.54	2.02 ± 0.60	n.s.
Viraemia (10^6 copies/ml)		5.15 ± 4.71	5.18 ± 4.81	n.s.
Histology				
Inflammatory grade		7.8 ± 2.5	7.9 ± 2.1	n.s.
Mean fibrosis stage		1.4 ± 0.2	1.3 ± 0.6	n.s.
Mean steatosis score		2.1 ± 0.3	2.1 ± 0.4	n.s.
Genotype				
1a		3	3	
1b		23	23	
2a		4	3	
3a		5	6	

(RIBA; Chiron Corporation, Emeryville, CA, USA). For hepatitis B virus serological markers, we used kits (Abbott Laboratories, Chicago, IL, USA). The presence of antibodies (anti-nuclear, anti-mitochondrial, anti-smooth muscle, anti- liver- kidney- microsome) was evaluated by means of indirect immunofluorescence.

Serum HCV RNA levels were measured by means of standardized quantitative PCR assay with a lower limit of detection of less than 1,000 copies/ml, using the Amplicor quantitative PCR system (Roche Diagnostic System Inc.-Branchburg, NJ, USA). Serum samples negative for HCV RNA were re-tested using a more sensitive standardized qualitative PCR assay with a lower limit of detection of about 100 copies/ml in order to confirm HCV-RNA disappearance.

HCV genotypes and subtypes were identified through a modification of the specific line probe assay (Inno-LiPA system; Innogenetics NV, Zwijnaarde, Belgium) as described by Stuyver et al. [19]. Briefly, primers complementary to the conserved sequences of the 5′ untranslated region of the different HCV genotypes were used in the reverse-transcription polymerase chain reaction (RT-PCR).

HCV RNA has been extracted from patients' sera and amplified by means of RT-PCR with the incorporation of biotinylated deoxyuridine triphosphate. Oligonucleotide probes (16-mers) specific for the different HCV genotypes and subtypes were hybridized with the patients' amplified viral complementary DNA.

Hybridisation was detected with ALP-labeled streptavidin and nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate chromagens. The HCV genotypes were designated according to the nomenclature proposed by Simmonds [20].

Histological findings

Liver biopsy was obtained using a modified Menghini technique. The specimen was fixed in neutral formaldehyde 4% solution for routine histological processing and

evaluation. The Knodell and Ishiak histological activity index (HAI) score was used to assess the histological grading of the disease [21].

All treated patients underwent a percutaneous liver biopsy in the 6 months before the onset of therapy and 6 months after the end of treatment. The inflammation score was obtained by combining scores for the first three components of the Knodell index: portal, periportal and lobular inflammation (range 0–18, with higher scores indicating more severe abnormalities); the fibrosis *stages* are: 0 = no fibrosis, 1 = portal fibrosis without septa; 2 = portal fibrosis with rare septa; 3 = numerous septa without cirrhosis; 4 = cirrhosis.

The severity of steatosis has been scored as follows: a score of 1 was assigned to the complete lack of lesions detectable in liver cells. Scores of 2 through 4 were assigned by assessing the percentage of hepatocytes containing fat droplets. Steatosis was considered as mild when involving less than 10% of hepatocytes and was graded as score 2; moderate when involving 10–30% of hepatocytes (score 3), and severe when involving more than 30% of hepatocytes (score 4) [22].

The pathologist was blinded to the treatment arms.

Statistical analysis

Means and standard deviations were used to describe the distribution of continuous variables. Two-sided Fisher's Exact Test was used to assess the differences in response rates at various time intervals after initiation of therapy in the two study groups. Histological differences between the paired initial and follow-up liver biopsy specimens were evaluated using the paired *t*-test. The primary endpoint of the study was to determine the efficacy of carnitine treatment in reducing hepatic steatosis in patients with HCV who were treated with IFN.

The null hypothesis was that there was no relationship between treatment and the differences in the histological score after treatment. The alternative hypothesis was that L-carnitine treatment would improve the steatosis score by 0.5 points in 40% of the patients, while non-treatment would result in a 0.5-point improvement in only 5% of the patients. To detect a 35% difference in efficacy with a power of 80% and a level of confidence of 95%, 18 subjects were required in each study group.

A goal of 35 patients enrolled in each arm of the study was planned to allow for dropout. All statistical tests were two sided with a level of significance of $P = 0.05$.

Efficacy and safety assessment

All enrolled patients were included in the intention-to-treat efficacy analysis, and patients who received at least one dose of IFNα plus ribavirin (1,000 mg) were included in the safety analysis. Data were analysed using an "intention to treat" principle. We considered patients as "end of treatment responders" (ETRs) when they showed a normalisation of serum ALT levels and a disappearance of serum HCV-RNA at the end of therapy. Sustained responders (SRs) were those who maintained this result during a 6-months follow-up period. The remaining patients were considered non-responders (NRs) or relapses. Adverse events were assessed by interviewing, and by means of laboratory and clinical examinations during treatment at weeks 1, 2 and 4 and then every month until the end of therapy. They were graded as mild, moderate and severe on the basis of the WHO score. The treatment should be definitively stopped in the case of severe events, such as hepatic failure, severe toxicity and no compliance; in moderate and mild cases, a dose reduction of 50% was performed until resolution of the event.

Results

Baseline characteristics

Baseline characteristics were evenly distributed across the two groups of enrolled patients. There were no significant differences between the two groups with regard to age, sex, time of exposure to virus C, ALT, AST, prothrombin time, total cholesterol, triglycerides, viraemia and genotypes or to mean inflammatory, fibrosis or steatosis scores.

Viral genotype 1 b was the most common in both groups (Table 1). Table 2 shows the mean values of the parameters examined at different stages of the planned protocol.

Comparison with baseline

In the group treated with IFN plus ribavirin, we found a significant decrease at the end of 6 months for the following parameters: AST and ALT serum levels [$P < 0.001$ (C.I. 8.58–55.02) and $P < 0.001$ (C.I. 32.04–77.76), respectively]. At the end of therapy in this same group, significant decreases were found for the following: AST and ALT serum levels [$P < 0.001$ (95% C.I. 30.83–76.37) and $P < 0.001$ (95% C.I. 43.35–92.25), respectively], and viraemia [$P < 0.01$ (95% C.I. 0.59–4.41)]. The same parameters were significantly decreased with respect to baseline values at the end of follow-up: AST [$P < 0.005$ (95% C.I. 13.30–56.50)], ALT [$P < 0.001$ (95% C.I. 29.72–79.08)], and viraemia [$P < 0.05$ (95% C.I. 0.16–4.12)]. We also observed a decrease in mean inflammatory score [$P = 0.07$ (95% C.I. –0.09–2.29)] and a significant decrease of mean fibrosis [$P < 0.005$ (95% C.I. 0.07–0.33)] (Table 3).

Table 2 Mean values of examined parameters (±SD) in the two arms at baseline, at months 6 and 12, and at the end of the follow-up period

		Group A (IFN α plus ribavirin)				Group B (IFNα + ribavirin + carnitine)			
		Baseline	6 months	12 months	Follow-up	Baseline	6 months	12 months	Follow-up
Aspartate aminotransferase	IU/I	116.0 ± 49.3	84.2 ± 40.1	62.4 ± 38.1	81.1 ± 32.6	125.0 ± 46.2	38.2 ± 7.1	48.5 ± 30.2	60.0 ± 37.1
Alanine aminotransferase	IU/I	156.0 ± 47.4	101.1 ± 40.8	88.2 ± 47.2	101.6 ± 48.1	162.0 ± 49.2	98.2 ± 37.4	67.8 ± 31.4	78.4 ± 28.8
Bilirubin	mmol/l	10.9 ± 8.7	10.4 ± 7.9	10.1 ± 7.6	10.2 ± 7.4	11.2 ± 9.1	10.9 ± 9.6	10.2 ± 7.9	10.5 ± 7.7
Albumin	g/dl	4.2 ± 0.3	4.2 ± 2.9	4.2 ± 0.3	4.2 ± 0.3	4.1 ± 0.3	4.1 ± 0.3	4.2 ± 0.3	4.1 ± 0.4
Total cholesterol	mmol/l	5.1 ± 0.9	5.1 ± 0.6	5.0 ± 0.7	5.0 ± 0.8	5.2 ± 0.6	4.8 ± 0.7	4.6 ± 0.8	4.7 ± 0.9
HDL	mmol/l	1.0 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.3
LDL	mmol/l	2.6 ± 0.7	2.3 ± 0.7	2.5 ± 0.6	2.5 ± 0.6	2.6 ± 0.7	2.5 ± 0.6	2.4 ± 0.5	2.4 ± 0.5
Triglyceride	mmol/l	2.2 ± 0.7	2.6 ± 0.7	2.4 ± 0.6	2.3 ± 0.7	2.2 ± 0.8	2.1 ± 0.7	2.0 ± 0.5	2.1 ± 0.5
Viraemia	10^6 copies/ml	5.1 ± 4.7	3.8 ± 2.4	2.6 ± 2.3	2.96 ± 2.7	5.1 ± 4.8	3.6 ± 2.1	2.5 ± 2.2	2.6 ± 2.4

Table 3 Liver histological features

	Group A (IFN α + ribavirin)		Group B (IFNα + ribavirin + carnitine)	
	Before	After	Before	After
Mean inflammation score	7.8 ± 2.5	6.7 ± 2.1	7.9 ± 2.1	6.3 ± 2.0
Mean fibrosis score	1.4 ± 0.2	1.2 ± 0.3	1.3 ± 0.4	1.0 ± 0.3
Steatosis score	2.2 ± 0.3	2.0 ± 0.2	2.1 ± 0.4	1.6 ± 0.3

In the group treated with IFN and ribavirin plus carnitine, we observed at the end of the 6 months a significant decrease with respect to baseline values for:

AST [$P < 0.001$ (C.I. 69.72–103.88)], ALT [$P < 0.001$ (C.I. 41.21–86.39)], total cholesterol [$P < 0.05$ (C.I. 0.06 to 0.74)] and viraemia [$P < 0.05$ (C.I. –0.41–3.41)] (Table 2). In the same group, we observed at the end of the treatment a significant decrease with respect to baseline values for: AST [$P < 0.001$ (95% C.I. 56.33–96.67)], ALT [$P < 0.001$ (95% C.I. 72.87–115.53)], total cholesterol [$P < 0.005$ (95% C.I. 0.20–0.98)] and viraemia [$P < 0.01$ (95% C.I. 0.67–4.53)] (Table 2). At follow-up we found a significant decrease for the following parameters: AST [$P < 0.001$ (C.I. 43.35–86.65)], ALT [$P < 0.001$ (C.I. 62.77–104.43)] and viraemia [$P < 0.05$ (C.I. 0.54–4.46)]. We observed a decrease in mean inflammatory score [$P < 0.05$ (C.I. 0.54–2.66)] and steatosis score [$P < 0.05$ (C.I. 0.04–0.40)].

Comparison between treatment groups

The comparison of treatments between the two groups showed a significant difference for the following parameters at 12 months: ALT –68 vs –84 IU/ml ($P < 0.001$; 95% C.I. –80.32 to –38.88), total cholesterol –0.08 vs –0.91 mmol/l ($P < 0.05$; 95% C.I. –0.01–0.83) and triglycerides +0.25 vs –20 mmol/l ($P < 0.05$; 95% C.I. –0.06–0.70); and at follow-up: AST –35 vs –65 IU/ml ($P < 0.05$; 95% C.I. 3.05–39.1), ALT –55 vs –84 IU/ml ($P < 0.05$; 95% C.I. 2.71–43.6), mean fibrosis –0.2 vs –0.3 ($P < 0.01$; 95% C.I. 0.04–0.3) and mean steatosis –0.1 vs –0.3 ($P < 0.005$; 95% C.I. 0.08–0.86). All values were lower in group B than A (Table 3).

The response at the end of treatment was 48% vs 56% and the SRs 39% vs 46% in groups A and B, respectively. We observed a decrease of fibrosis in 33% and 67% and a decrease of steatosis in 30% and 70% in groups A and B, respectively.

Adverse events

No serious adverse events (World Health Organization grade 3 or 4) were reported in the two groups. However, six patients from group A and two from group B complained of mild psychological disorders such as anxiety, irritability and depression.

Ribavirin is known to accumulate in red cells and provoke haemolysis. Median haemoglobin concentration fell significantly during the first 3 months of treatment in both groups, remaining stable for 3 months and returning to values similar to baseline within 3 months after the end of

the treatment. Noteworthy, a higher decrease of haemoglobin values was observed in the IFN plus ribavirin alone treatment. The patients who also received carnitine treatment experienced a fall in median haemoglobin concentration from 13.8 g/dl (range 12.0–15.6 g/dl) to 11.8 (range 10.2–14.6 g/dl) at the end of therapy. The decrease in those from the other group with no carnitine was from 13.4 g/dl (range 11.4–16.9) to 11.2 g/dl (range 10.1–13.5 g/dl).

Significant decreases in the white cell blood count were observed in the group receiving IFN plus ribavirin . The platelet counts did not significantly change in either group. Other side effects registered in both groups were anorexia (13% in group A and 8% in group B), nausea (20 and 14% respectively), weight loss (14 and 5%), headache (54 and 28%), fatigue (52 and 35%), myalgia (40 and 18%), musculoskeletal pain (36 and 12%) and irritability (18 and 8%).

Most patients in this clinical registration trial managed to achieve the goals of 64% adherence to their medication dose and duration of therapy.

Discussion

A recent study showed that prolonged treatment with IFNα plus ribavirin represents the better response in patients with chronic HCV-related hepatitis. Liver steatosis may contribute to progression of fibrosis and reduction of the effectiveness of specific antiviral therapies in such patients. Carnitine activity in the pathogenetic steps of steatosis now represents a new therapeutic opportunity to improve antiviral therapies.

The combination of carnitine and IFNα plus ribavirin seems more efficacious than IFNα plus ribavirin alone in the treatment of chronic active hepatitis C, as suggested by SRs at the end of follow-up in this study. Carnitine is found in high concentrations in leucocytes, and it is envisaged that carnitine is involved in regulating the immune response [23–25].

Several data suggest an interaction between HCV and lipid metabolism. HCV is associated with serum low beta-lipoproteins of infected patients [26], and the mechanisms leading to lipid accumulation in the hepatocytes of HCV-infected patients appear multifactorial.

A metabolic type of steatosis is frequently seen in patients with HCV1 or HCV2, while the viral type is typically seen with HCV-3 and might be related to the direct effect of viral proteins, which interfere with the intracellular uptake and transport of triglycerides and with the assembling and secretion of lipoproteins [27–29]. Several previous studies have demonstrated that IFN therapy is associated with a decrease in mean serum cholesterol levels, although the mechanism of this action is still unclear [30–32]. We hypothesized that the cholesterol decrease is due to the antiviral action of IFN. In fact, the decrease of viral load in serum induces an increased deliverance of LDL-C receptors, enhancing for consequence the cholesterol metabolism. The carnitine, due to its role on energetic mitochondrial metabolism, could improve the intracellular processing of cholesterol, making it less toxic.

Carnitine administration has been demonstrated to reduce blood and tissue lipid accumulation in various conditions, including cardiovascular disease, chronic alcoholism and dietary carnitine deficiency [33, 34]. Furthermore, we observed a significant increase in mean serum triglyceride levels during therapy with IFN for chronic hepatitis C [11]. Richter et al. reported that administration of L-carnitine reduced the sucrose-induced hypertriglyceridaemia and the increase of free fatty acid levels in rat plasma [35]. Carnitine supplementation of semi-starved rats significantly increased the activity of preheparin plasma lipoprotein lipase and restored plasma triacylglycerol secretion rate to the normal level [36].

IFNs inhibit lipoprotein lipase and hepatic triglyceride lipase, but they may also stimulate hepatic lipogenesis [12, 13]. The exogenous IFNα administration enhances the synthesis and release of cytokines (interleukin 1, interleukin 6, TNF), which are produced by monocytes and macrophages [37]. Both tumour necrosis factor and interleukin 1 can increase serum triglyceride levels through stimulation of hepatic lipogenesis [38]. The carnitine is also active on triglycerides by inducing lipidic peroxidation as well as proteic acylation and de-acylation (VLDL at endoplasmic reticulum level).

Carnitine exerts its role in some of the pathogenetic steps of steatosis: the action on triglycerides and mitochondria allows the utilization of the substrates and free fatty acids reducing their amount within the hepatocytes. Free fatty acids are able to produce damage on cell and mitochondrial surfaces. Microvesicular fatty change is the result of an impaired β-oxidation, consequent to the mitochondrial damage. In a murine model of fatty change, both acute and chronic fatty changes were associated with lipid peroxidation disturbances [39]. The carnitine plays an important role in the mitochondrial uptake of long chain fatty acids by facilitating their transportation across the inner mitochondrial membrane to undergo β-oxidation, and it also affects glucose metabolism by activating pyruvate dehydrogenase [40–42]. Carnitine transfers long-chain acyl-CoAs to produce short-chain acylcarnitine, which can be shuttled out of mitochondria. IFN has been described as a possible factor of steatosis because it inhibits the transcription of mitochondrial DNA into mitochondrial messenger RNA [43].

The limitations of this study include the relatively small sample size and its open label design. However, as the

efficacy end points of the study revolve around measurements of ALT, AST, viraemia and liver biopsy, the non-blinded nature of the study was unlikely to have caused significant bias. A further limitation of the study is that there was no subanalysis performed for different HCV genotypes. Steatosis was presumed to be a cytopathic effect of HCV genotype 3; although, in HCV type-1-infected patients, steatosis is mainly related to metabolic factors. It would be interesting to investigate whether adjuvant carnitine treatment would result in a different outcome according to genotype.

Although carnitine is also known to be a radical scavenger [44], it has been suggested that L-carnitine interferes with oxygen free radical (ROS) formation [45, 46], reducing the generation of elevated amounts of ROS. ROS production can exceed cellular antioxidant defence capabilities and can result in severe metabolic dysfunction, including peroxidation of lipid membrane and mitochondrial damage [47].

In patients with steatosis, histological improvement is very uncommon.

In conclusion, the results of this study provide evidence that L-carnitine supplementation can stimulate fatty acid metabolism and change the lipid profile of serum. L-Carnitine treatment may decrease liver steatosis in patients with hepatitis C treated with IFNα and ribavirin.

References

1. Barba G, Harper F, Harada T, Kohara M, Goulinet S, Matsuura Y, Eder G, Schaff Z, Chapman MJ, Miyamura T, Brechot C (1997) Hepatitis C Virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets. Proc Natl Acad Sci U S A 94:1200–1205
2. Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura Y, Miyamura T, Koike K (1997) Hepatitis C Virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol 78:1527–1531
3. Garcia-Monzon C, Martin-Perez E, Iacono OL, Fernandez-Bermejo M, Majano Pl, Apolinario A, Larranaga E, Moreno-Otero R (2000) Characterization of pathogenetic and prognostic factors of non-alcoholic steatohepatitis associated with obesity. J Hepatol 33:716–724
4. Czaya AJ, Carpenter HA, Santrach PJ, Moore SB (1998) Host- and disease-specific factors affecting steatosis in chronic hepatitis C. J Hepatol 29:198–206
5. Bach N, Thung SN, Shaffner F (1992) The histological features of chronic hepatitis C and autoimmune chronic hepatitis: a comparative analysis. Hepatology 15:572–577
6. Ratziu V, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T (2000) Liver fibrosis in overweight patients. Gastroenterology 118:1117–1123
7. Angulo P, Keach JC, Batts KP, Lindor KD (1999) Independent predictors of liver fibrosis in patients with non-alcoholic steatohepatitis. Hepatology 30:1356–1362
8. Alter MJ, Kruszon-Moran D, Nainan OV, Mcquillan GM, Gao F, Moyer LA, Kaslow RA, Margolis HS (1999) The prevalence of hepatitis C virus infection in the United States 1988 through 1994. N Engl J Med 341:556–562
9. Lauer GM, Waker BD (2001) Hepatitis C Virus infection. N Engl J Med 345:41–52
10. Malaguarnera M, Giugno I, Trovato BA, Panebianco MP, Siciliano R, Ruello P (1995) Lipoprotein (A) concentration in patients with chronic active C before and after interferon treatment. Clin Ther 17:721–728
11. Malaguarnera M, Giugno I, Ruello P, Pistone G, Restuccia S, Trovato BA (1996) Effects of interferon on blood lipids. Clin Drug Invest 11:43–48
12. Naeem M, Bacon BR, Mistry B, Britton RS, Di Bisceglie AM (2001) Changes in serum lipoprotein profile during interferon therapy in chronic hepatitis C. Am J Gastroenterol 96:2468–2472
13. Rebouche CJ, Seim H (1998) Carnitine metabolism and its regulation in microorganisms and mammals. Ann Rev Nut 18:39–61
14. Romano M, Malaguarnera M, Vinci M, Batticani S, Consoli G, Navarria G (1996) Incidence and ultrasonographic patterns of hepatic steatosis in the elderly. Arch Gerontol Geriatr 5:313–316
15. Malaguarnera M, Restuccia N, Di Fazio I, Panebianco MP, Gulizia G, Giugno I (1999) Fish oil treatment of interferon-alpha-induced dyslipidemia: study in patients with chronic hepatitis C. Biodrugs 11:285–291
16. Malaguarnera M, Maugeri D, Saraceno B, Romano M, Neri S, Rapisarda R, Pistone G (2002) Effects of carnitine on biochemical responses in patients with chronic hepatitis C treated with interferon-A. Clin Drug Invest 22:443–448
17. Malaguarnera I, Di Fazio S, Restuccia G, Pistone N, Restuccia BA (1998) Trovato efficacy of different schedules in the management of chronic hepatitis C with interferon-α. Ann Med 30:213–217
18. Alberti A, Benvegnu L (2003) Management of hepatitis C. J Hepatol 38:S104–118
19. Stuyver L, Wyseur A, Van Arnhem W, Hernandez F, Maertens G (1996) Second-generation line probe assay for hepatitis C virus genotyping. J Clin Microbiol 34:2259–2266
20. Simmonds P, Alberti P, Halter HJ, Bonino F, Bradley DW, Brechot C, Brouwer JT (1994) A proposed system for the nomenclature of hepatitis C viral genotypes. Hepatology 19:1321–1324
21. Knodell R, Ishack K, Black W (1981) Formulation and application of numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1:431–435
22. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR (1999) Non-alcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 94:2467–2474
23. Deufel T (1990) Determination of L-carnitine in biological fluids and tissues. J Clin Chem Clin Biochem 28:307–311
24. Malaguarnera L, Rosa MD, Zambito AM, Dell'ombra N, Marco RD, Malaguarnera M (2006) Potential role of chitotriosidase gene in nonalcoholic fatty liver disease evolution. Am J Gastroenterol 101(9):2060–2069 Epub 2006 Jul 18
25. Malaguarnera L, Di Rosa M, Zambito Am, Dell'ombra N, Nicoletti F, Malaguarnera M (2006) Chitotriosidase gene expression in Kupffer cells from patients with non-alcoholic fatty liver disease. Gut 55(9):1313–1320 Epub 2006 Jul 6
26. Thomssen R, Bonk S, Propfee C, Heerman KH, Kochel HG, Uy A (1992) Association of hepatitis C virus in human sera with beta lipoprotein. Med Microbiol Immunol 181:293–300
27. Rubbia-Brandt L, Quadri R, Abid K, Giostra E, Male PJ, Mentha G, Spahr L, Zarski JP, Borisch B, Hadengue A, Negro F (2000) Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype. J Hepatol 33:106–115

28. Kumar D, Farrell GC, Fung C, George J (2002) Hepatitis C virus genotype 3 is cytopathic to hepatocytes: reversal of hepatic steatosis after sustained therapeutic response. Hepatology. 36:1266–1272
29. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G (2001) Steatosis accelerates the progression of liver damage of chronic hepatitis c patients and correlates with specific hcv genotype and visceral obesity. Hepatology 33:1358–1364
30. Shinohara E, Yamashita S, Kihara S, Hirano K, Ishigami M, Arai T, Nozaki S, Kameda-Takemura K, Kawata S, Matsuzawa Y (1997) Interferon alpha induces disorder of lipid metabolism by lowering postheparin lipases and cholesteryl ester transfer protein activities in patients with chronic hepatitis C. Hepatology 25:1502–1506
31. Dixon RM, Borden EC, Keim NL, Anderson S, Spennetta TL, Tormey DC, Shrago E (1984) Decreases in serum high-density lipoprotein cholesterol and total cholesterol resulting from naturally produced and recombinant DNA-derived leukocyte interferons. Metabolism 33:400–404
32. Massaro ER, Borden EC, Hawkins MJ, Wiebe DA, Shrago E (1986) Effects of recombinant interferon-alpha 2 treatment upon lipid concentrations and lipoprotein composition. J Interferon Res 6:655–665
33. Maebashi M, Kawamura N, Sato M, Imamura A, Yoshinaga K (1978) Lipid-lowering effect of carnitine in patients with type-IV hyperlipoproteinaemia. Lancet 2:805–807
34. Khan L, Bamji MS (1979) Tissue carnitine deficiency due to dietary lysine deficiency: triglyceride accumulation and concomitant impairment in fatty acid oxidation. J Nutr 109:24–31
35. Richter V, Rassoul F, Schulz G, Sittner WD, Seim H, Loster H, Rotzsch W (1987) Carnitine and experimental carbohydrate-induced hyperlipoproteinemia. Arch Int Pharmacodyn Ther 290(1):138–144
36. Feng Y, Guo C, Wei J, Yang J, Ge Y, Gao L (2001) Necessity of carnitine supplementation in semistarved rats fed a high-fat diet. Nutrition 17(7–8):628–631
37. Malaguarnera M, Pistone G, Neri S, Romano M, Brogna A, Musumeci S (2004) Interleukin-2 plus ribavirin versus interferon-alpha-2b plus ribavirin in patients with chronic hepatitis C who did not respond to previous interferon-alpha-2b treatment. Biodrugs 18:407–413
38. Famularo G, De Simone C (1995) A new era for carnitine? Immunol Today 16:211–213
39. Sougero C, Joo M, Chianese-Bulock KA, Nguyen DT, Tung K, Hahn V (2002) Hepatitis C virus core protein leads to immune suppression and liver damage in a transgenic murine model. J Virol 76:9345–9354
40. Lederblad G (1976) Plasma carnitine and body composition. Clin Chim Acta 67:207–212
41. Lysiak W, Lilly K, Dilisa F, Toth PP, Bieber LL (1988) Quantitation of the effect of L-carnitine on levels of acid-soluble short-chain acyl-CoA and CoASH in rat heart and liver mitochondria. J Biol Chem 263:1151–1155
42. Feng V, Suo CJ, Wei JV et al (2000) The impact of fat rich feeding on carnitine and lipid metabolism in half-starved rats. Chin J Clin Nutr 8:96–101
43. Lewis JA, Huq A, Najarro P (1996) Inhibition of mitochondrial function by interferon. J Biol Chem 271:13184–13190
44. Kraemer WJ, Volek JS, French DN, Rubin MR, Sharman MJ, Gomez AL, Ratamess NA, Newton RU, Jemiolo B, Craig BW, Hakkinen K (2003) The effects of L-carnitine L-tartrate supplementation on hormonal responses to resistance exercise and recovery. J Strength Cond Res 17(3):455–462. Pmid: 12930169
45. Fernandez-Checa JC, Kaplowitz N, Garcia-Ruiz C, Colell A, Miranda M, Mari M, Ardite E, Morales A (1997) Gsh transport in mitochondria: defense against tnf-induced oxidative stress and alcohol-induced defect. Am J Physiol 273(1 Pt 1):G7–17. Review. Pmid: 9252504
46. Quillet-Mary A, Jaffrezou JP, Mansat V, Bordier C, Naval J, Laurent G (1997) Implication of mitochondrial hydrogen peroxide generation in ceramide-induced apoptosis. J Biol Chem 272(34):21388–21395. Pmid: 9261153
47. Perez-Carreras M, Del Hoyo P, Martin MA, Rubio JC, Martin A, Castellano G, Colina F, Arenas J, Solis-Herruzo JA (2003) Defective hepatic mitochondrial respiratory chain in patients with non-alcoholic steatohepatitis. Hepatology 38:999–1007