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Original Article

Effect of L-carnitine on DNA damage and oxidative stress in maintenance hemodialysis patients with hepatitis C virus infection in East China

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Abstract: Objectives: L-carnitine has been used as therapy in hemodialysis patients for many years. When HCV was present, higher rates of patient mortality displayed. However, no study is concerned with the synergistic effect of L-carnitine and HCV. The purpose of this study was to investigate the effect of L-carnitine on the markers of DNA damage and oxidative stress in maintenance hemodialysis patients with HCV infection. Material and methods: The blood samples were collected from 63 patients from March 2011 to November 2013 (43 HCV-positive patients in hemodialysis and 20 HCV-negative patients on dialysis). DNA damage was evaluated by cytokinesis-block micronucleus cytome (CBMN-Cyt) assay. Oxidative stress indicators (superoxide anion, hydrogen peroxide, hydroxyl radical, malondialdehyde, superoxide dismutase, glutathione) were determined with the kits. Results: The results showed that L-carnitine had no effect on the levels of free radical indicator, free radical damage indicator and endogenous antioxidant enzymes. The frequencies of micronuclei (MN) and nuclear buds (NBUD) were remarkably reduced after the treatment of L-carnitine. Conclusion: Taken together, our findings indicate that L-carnitine supplementation reduced oxidative DNA damage in peripheral blood lymphocytes of maintenance hemodialysis patients with HCV infection. The patients might benefit from L-carnitine supplementation by inhibiting DNA damage.

Keywords: HCV, hemodialysis, L-carnitine, DNA damage, oxidative stress

Introduction

During daily medical procedure, HCV and chronic renal disease are commonly appeared. And these two diseases are potentially serious medical problems throughout the world. Recently, some scientists found that these two diseases are potentially closely connected during many aspects. Patients with end-stage renal disease (ESRD) on maintenance hemodialysis are at increased risk for acquiring HCV infection because of prolonged vascular access and potential for exposure to contaminated equipment [1]. Therefore, there is an urgent need to seek novel ways for therapy of HCV-infected hemodialysis patients.

L-carnitine is an essential nutrient that the body uses to convert fat into energy, which participates in metabolism of branched chain amino acids, and stabilizes cellular membranes [2]. It is also an antioxidant that reduces metabolic stress in the cells. Studies have reported that L-carnitine have an effective free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and total reducing power [3]. It prevents the formation of reactive oxygen species (ROS) and protects cells from peroxidative stress. Also, it has a lot of benefits in many physiopathological processes.

Recent years, L-carnitine has been proposed for treatment of various kinds of disease, including liver injury [4, 5], coronary artery disease [6] and ESRD [7]. Molyneux R et al [7] demonstrated that myocardial carnitine is significantly depleted particularly in uraemic patients and that on dialysis therapy; carnitine treatment has cardiovascular benefits including

Table 1. Characteristics of subjects[1]

	Control (n=20)	L+ (n=22)	L- (n=21)	*P*-value[2]
Gender (male)	10	11	11	-
Age (y)	55.9±14.5	57.2±10.7	50.5±16.1	0.27
TBIL (μmol/L)	6.2±1.9	6.6±2.0	6.5±2.5	0.91
DBIL (μmol/L)	2.5±0.7	2.9±0.5	2.7±1.0	0.48
AST (IU/L)	12.9±3.5	18.6±11.0	17.7±9.0	0.85
ALT (IU/L)	7.2±1.9	10.3±3.6	10.2±3.0	0.97
HCRP (mg/L)	1.8±1.5	1.3±1.0	1.5±1.1	0.63

[1]Mean ± SD, [2]values show no obvious differences between L+ and L- group.

modulation of myocardial metabolism, reduction in necrotic cell death and infarct size. Boyacioglu M et al [8] reported that L-carnitine may have a preventative effect in alleviating the negative effects of contrast-induced nephropathy (CIN). However, information regarding the effect of L-carnitine in hemodialysis patients with HCV infection is little, and further research is required.

In this study, our work undertaken was to evaluate the protective effect of L-carnitine on DNA damage and oxidative stress in maintenance hemodialysis patients with HCV infection. DNA damage was detected by cytokinesis-block micronucleus cytome (CBMN-Cyt) assay that measures chromosomal DNA damage, cytostasis and cytotoxicity events at the single cell level. The level of generation of ROS (superoxide anion O_2^-, hydrogen peroxide H_2O_2, hydroxyl radical OH), lipid peroxidation product (malondialdehyde MDA) and consumption of endogenous antioxidant enzymes (superoxide dismutase SOD, glutathione GSH) were determined with the kits.

Materials and methods

Subjects

The Affiliated Yixing Hospital of Jiangsu University Committee approved all procedures associated with this study. Written informed consent was obtained from all the participants. The patients receiving antioxidants including Vitamin C, Vitamin E, edaravone, ebselen, resveratrol and other Chinese herbs which literature implying antioxidant property, were excluded. The venous blood samples were collected from 43 HCV-positive patients on hemodialysis and 20 HCV-negative patients on dialysis in the Affiliated Yixing Hospital of Jiangsu University from March 2011 to November 2013. The median age of patients (27 men and 36 women) was 61 years (range 43-83 years). The serum samples were obtained after centrifugation (1,000 g, 4°C, 15 minutes) and stored at -80°C until analysis.

Study protocol

This study was designed as a single blind, randomized, parallel, placebo-controlled trial. All the participants were divided into three groups: HCV-positive patients on hemodialysis treated with L-carnitine (L+ group, n=22), HCV-positive patients on hemodialysis without treatment of L-carnitine (L- group, n=21) and HCV-negative patients on dialysis treated with L-carnitine (control group, n=20). L-carnitine was purchased from Sigma-Tau (Rome, Italy). Patients were used by infusion 5 mg/kg/d. The L-carnitine infused to the patients for at least 1 month to maintain the plasma concentration.

Cytokinesis-block micronucleus cytome (CBMN-Cyt) assay

A half milliliter of whole blood is added to 4.5 ml of RPMI 1640 culture medium, phytohemagglutinin (PHA) was also added to stimulate the lymphocytes. Cytochalasin B (6 μg/mL, Sigma Chemical Co., St Louis, MO) was added after 44 h of culture to block cytokinesis, which allowed to identify proliferating lymphocytes in cell culture. Cells that have undergone the first mitosis are thus recognized as binucleated cells and are selectively screened for the presence of micronuclei (MN), nucleoplasmic bridges (NPB), and nuclear buds (NBUD) [9]. Cell harvesting, hypotonic treatment, fixation, and slide preparation were performed according to standard procedures. MN was scored blindly in 1,000 binucleated cells, following standard criteria, and the frequency was expressed as the number of binucleated cells presenting one or more MN/NPB/NBUD per 1,000 cells.

Biochemical analysis

In this study, oxidative stress indicators were divided into three categories: Free radical indicator (superoxide anion, hydrogen peroxide, hydroxyl radical), Free radical damage indicator (malondialdehyde MDA), endogenous antioxidants indicator (Superoxide dismutase, gluta-

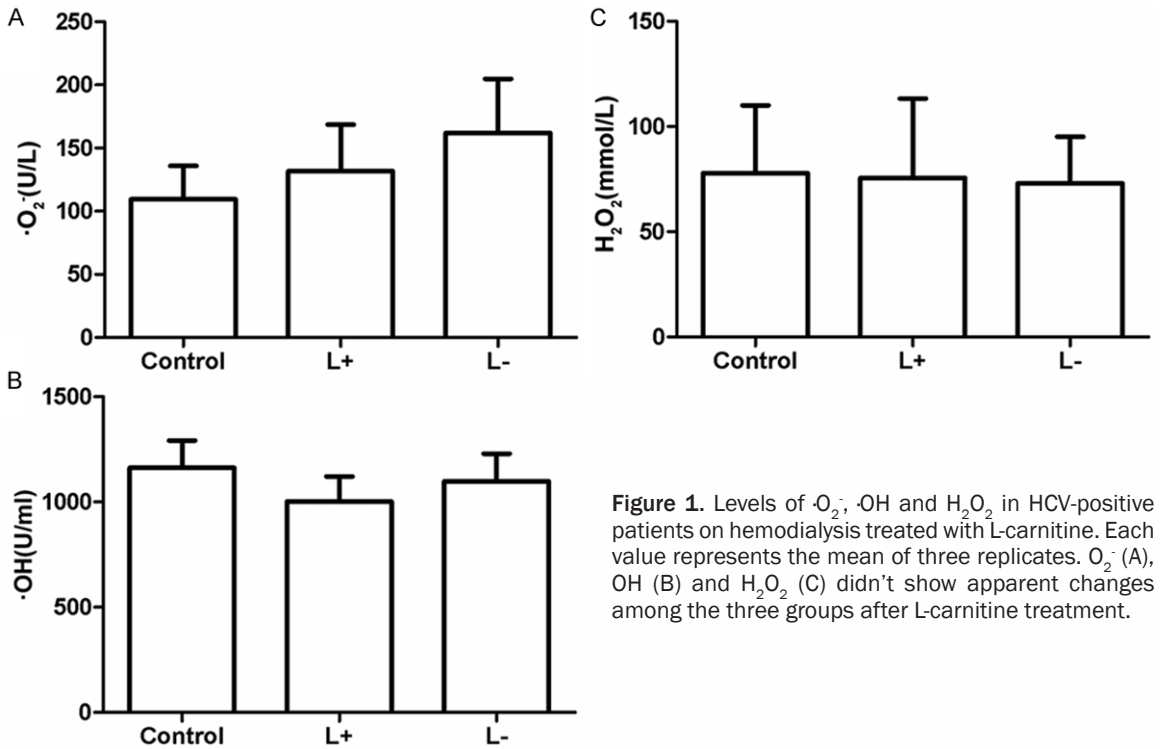

Figure 1. Levels of $\cdot O_2^-$, ·OH and H_2O_2 in HCV-positive patients on hemodialysis treated with L-carnitine. Each value represents the mean of three replicates. O_2^- (A), OH (B) and H_2O_2 (C) didn't show apparent changes among the three groups after L-carnitine treatment.

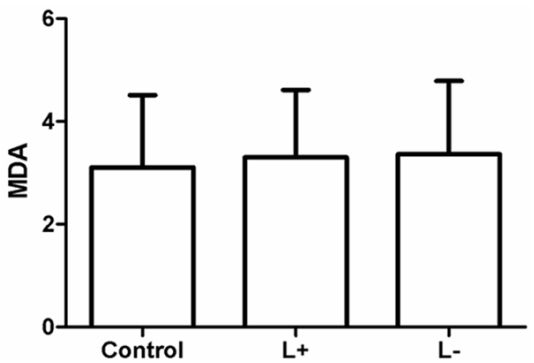

Figure 2. Effect of L-carnitine on the expressions of MDA in HCV-positive patients on hemodialysis. Data were presented as mean ± SD of three replicates. There were no significant changes in MDA levels after L-carnitine supplement.

thione). All the kits were purchased from Jiancheng Bioengineering Research Institute (Nanjing, China) and all procedures were done according to the manufacturer's instructions.

Statistical analysis

The data were analyzed using GraphPad Prism 5 (Graphpad Software Inc, San Diego, CA). Statistical analysis was performed by t test. Data are presented as mean ± standard deviation (SD). Statistical *P* values <0.05 were considered significant.

Results

Study participant characteristics

Table 1 shows the demographic data and health characteristics of the participants. There were no significant differences among the three groups with respect to gender, age, the level of total bilirubin (TBIL), direct bilirubin (DBIL), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and high-sensitivity C-reactive protein (HCRP).

Effects of L-carnitine on the levels of free radical indicator, free radical damage indicator and endogenous antioxidant enzymes

To explore the levels of oxidative stress, free radical indicator (superoxide anion O_2^-, hydrogen peroxide H_2O_2, hydroxyl radical OH) were determined. The results revealed that all the three indicators didn't show any significant changes among the three groups (**Figure 1**). As shown in **Figure 2**, the levels of MDA showed similar panel with free radical indicators. Further, consistent results were obtained when we detected the levels of two endogenous antioxidant enzymes (superoxide dismutase SOD, glutathione GSH-Px). There were no obvious differences of SOD and GSH-Px among the three groups (**Figure 3**). In brief, these findings sug-

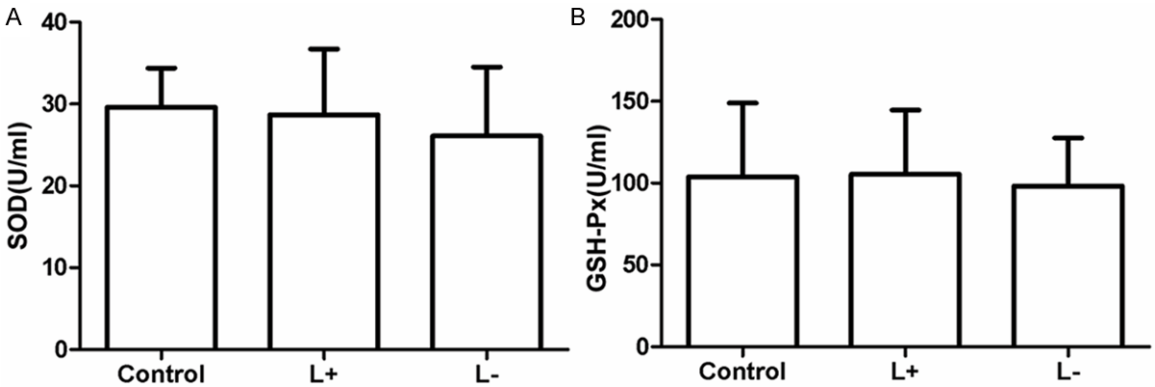

Figure 3. Levels of antioxidant enzymes (SOD and GSH-Px) activities after L-carnitine treatment. Each value represents the mean of three replicates. No obvious differences of SOD (A) and GSH-Px (B) were observed among the three groups.

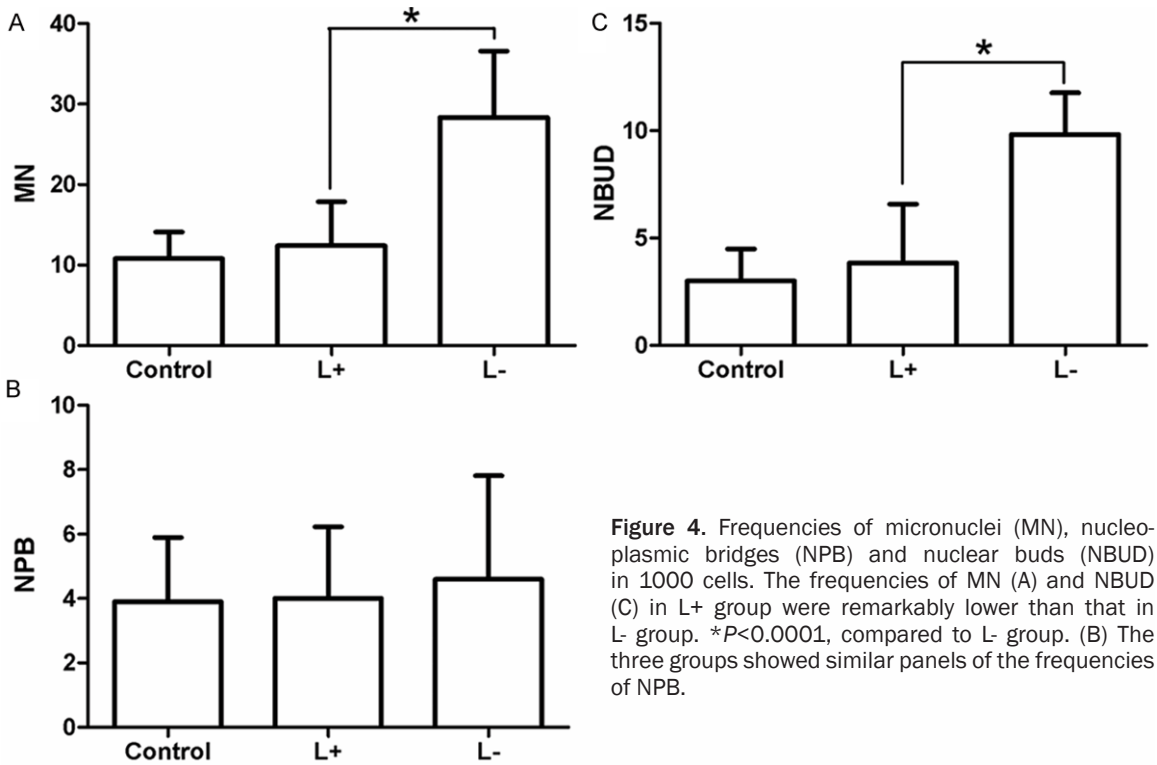

Figure 4. Frequencies of micronuclei (MN), nucleoplasmic bridges (NPB) and nuclear buds (NBUD) in 1000 cells. The frequencies of MN (A) and NBUD (C) in L+ group were remarkably lower than that in L- group. **P*<0.0001, compared to L- group. (B) The three groups showed similar panels of the frequencies of NPB.

gest that L-carnitine had no effect on the levels of free radical indicator, free radical damage indicator and endogenous antioxidant enzymes.

Chromosome instability of lymphocytes in the patients

In order to evaluate the DNA damage of the lymphocytes from the patients' blood, we conducted the CBMN assay to detect the frequencies of micronuclei (MN), a biomarker of chromosome loss and/or breakage, nucleoplasmic bridges (NPB), a biomarker of DNA misrepair and/or telomere end fusions and nuclear buds (NBUD), a biomarker of elimination of amplified DNA and/or DNA repair complexes [9]. As revealed in **Figure 4A** and **4C**, the frequencies of MN and NBUD in L+ group were remarkably lower than that in L- group, while there were no significant differences of MN and NBUD between the control and L+ group. And it's worth noting that the frequencies of NPB showed no obvious change among the three groups (**Figure 4B**). Thus, our results showed

that L-carnitine supplementation reduced oxidative DNA damage in peripheral blood lymphocytes of maintenance hemodialysis patients with HCV infection.

Discussion

It was previously demonstrated that hemodialysis patients exhibit a high incidence of cardiovascular pathologies and different types of cancer (mainly cervical, bladder, thyroid, and renal cell carcinoma) [10] as well as enhanced genetic damage [11-13], leading to an increased mortality. These high levels of genetic damage can be caused by the genomic instability that these patients show as consequence of the pathology [14]. Patients on maintenance hemodialysis are at increased risk for acquiring HCV infection [15]. The major concern is the lack of safe and effective drugs to treat HCV-infected hemodialysis patients. Therefore, there is an urgent need to seek for novel ways to diminish the levels of genetic damage in circulating lymphocytes.

Since oxidative DNA damage can be considered a biomarker of genomic instability, we evaluated oxidative DNA damage using the CBMN-Cyt assay. The CBMN-Cyt assay is well validated on chromosomal DNA damage and has been widely used in human diseases, such as cardiovascular disease [16], neurodegenerative disease [17], obstructive sleep apnea hypopnea syndrome [18] and Alzheimer's disease [19]. However, the CBMN-Cyt assay approach has not been previously applied to the lymphocytes of HCV-infected hemodialysis patients. It was therefore important to test whether chromosomal damage biomarkers were altered in those HCV-positive hemodialysis patients after L-carnitine treatment. In the current study, we have demonstrated that L-carnitine supplementation could reduce oxidative DNA damage in peripheral blood lymphocytes of HCV-infected hemodialysis patients. As a result, it seems clear that L-carnitine has a protective effect on HCV-positive hemodialysis patients, which could be ascribed to the modulation of DNA repair by L-carnitine. It is notable that our results displayed a reduction of MN and NBUD in the lymphocytes of HCV-positive hemodialysis patients after L-carnitine treatment, but no significant change in NPB. MN, NPB and NBUD are all biomarkers of genotoxic events and chromosomal instability [20]. Nevertheless, the mechanisms that lead to MN, NPB and NBUD formation are not well understood. According to our results, L-carnitine is an effective treatment for HCV-positive hemodialysis patients by the downregulation of MN and NBUD. The molecular mechanisms leading to MN, NBUD alternation still needs to be further investigated.

Reactive oxygen species (ROS) are considered to be involved in maintenance hemodialysis patients. Previous studies have confirmed that L-carnitine is an antioxidant [3]. Dobrzyńska I et al found that L-carnitine protected liver cell membranes against oxidative modifications in ethanol intoxicated rats through its ability to scavenge free radicals [21]. Thus, we speculated that antioxidant activity of L-carnitine may also play a role in the treatment of HCV-infected hemodialysis patients. However, the results from our studies clearly showed that L-carnitine had no effect on the levels of oxidative stress markers (O_2^-, H_2O_2, OH, MDA, SOD, GSH). Reactive oxygen species are known to be increased in HD patients. Moreover, previous studies have shown that oxidative stress is present in patients with chronic hepatitis C than those in other inflammatory liver disease [22]. Further, HCV core protein induces the production of reactive oxygen species [23, 24]. We thought the reason of discrepancy between our work and previous studies may be that the dose of L-carnitine was not high enough to remarkably reduce extremely high level of oxidative stress in hemodialysis patients with HCV infection.

There remain several limitations of the present study that should be mentioned. First, the number of participants was small, although we did recruit more subjects than expected. Second, the mechanistic explanation for the change of MN and NBUD frequencies after L-carnitine treatment in HCV-positive hemodialysis patients is still unclear.

Conclusion

In conclusion, our findings indicate that L-carnitine supplementation reduced oxidative DNA damage in peripheral blood lymphocytes of maintenance hemodialysis patients with HCV infection. Further studies will be undertaken to explore the antioxidative DNA damage mechanism of L-carnitine in these patients. Moreover, a follow-up study will also be con-

ducted to assess whether L-carnitine could improve prognosis of hemodialysis patients with HCV infection.

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Disclosure of conflict of interest

None.

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