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ORIGINAL PAPER

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Effects of doxorubicin-containing chemotherapy and a combination with L-carnitine on oxidative metabolism in patients with non-Hodgkin lymphoma

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Abstract *Purpose*: Chemotherapy regimens based on anthracycline (doxorubicin) are well established in lymphoma therapy. The purpose of this study was to examine the effects of L-carnitine with a view to reducing cytotoxic side-effects. *Methods*: 20 patients were scheduled to receive 3 g L-carnitine before each chemotherapy cycle, followed by 1 g L-carnitine/day during the following 21 days, while 20 patients received a placebo (randomized controlled trial). The plasma lipid profile and relative mRNA levels of key enzymes of oxidative metabolism (carnitine acyltransferases) were measured at three points of time. In addition to the clinical parameters we used the mRNA of white blood cells to evaluate the toxic effects on cardiomyocytes. *Results*: In the present study no cardiotoxicity of anthracycline therapy was detected. Carnitine treated patients showed a rise in plasma carnitine which led to an increase of relative mRNA levels from CPT1A (liver isoform of carnitine palmitoyltransferase) and OCTN2 (carnitine transporter). Following chemotherapy, an activation of carnitine acyltransferases was associated with a stimulation of OCTN2 in both groups. *Conclusion*: Biochemical and molecular analyses indicated a stimulation of oxidative metabolism in white blood cells through carnitine uptake.

Keywords Anthracycline · L-Carnitine · Oxidative metabolism

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Introduction

For more than two decades anthracycline doxorubicin (adriamycin, ADR) has been a constituent of the most effective chemotherapeutic strategies (Hale and Lewis 1994; Rabbani et al. 2005) in the treatment of a variety of malignancies, including lymphoma, leukemia, and solid tumors. Unfortunately the dose-dependent cardiotoxicity of doxorubicin limits its clinical use and threatens the cardiac function of many patients with cancer. It has been suggested that doxorubicin may exert at least part of its effect by inhibiting fatty acid (FA) oxidation in the heart (Sayed-Ahmed et al. 2000; Yoon et al. 2003). Free fatty acids (FFAs) represent the major source of energy in the normal adult working heart, whereas inhibition of their metabolism results in the accumulation of toxic intermediates. One strategy for the prevention of anthracycline-induced cardiotoxicity is based on the use of L-carnitine (β-hydroxy-γ-trimethylaminobutyric acid). Carnitine is a compound known for its function on the transport of long-chain FAs into the mitochondrial matrix, where the fatty acyl group is metabolized. Novel aspects of L-carnitine were recently summarized (Pittner et al. 2005). Carnitine acyltransferases, in particular carnitine palmitoyltransferase-1 (CPT1), play a key role in fatty acid oxidation. Since depletion of L-carnitine has been associated with the inhibition of CPT1 in cardiac tissue after adriamycin

treatment, it was speculated that the doxorubicin-induced apoptosis of cardiac myocytes is mediated by decreased CPT-dependent fatty acid oxidation.

In vitro studies showed that low carnitine levels in the culture media resulted in the downregulation of CPT1 and carnitine acetyltransferase (CRAT). Carnitine administration restored the mRNA expression in a dose-dependent manner (Lohninger et al. 2002). Since depletion of L-carnitine has been associated with the inhibition of CPT1 in cardiac tissue after adriamycin treatment, it was speculated that the doxorubicin-induced apoptosis of cardiac myocytes is mediated by decreased CPT-dependent fatty acid oxidation. Inhibition of CPT1 has recently been reported as inducing programmed cell death in some cell systems (Paumen et al. 1997a, b). This phenomenon was linked to the generation of ceramide (Andrieu-Abadie et al. 1999).

In rats ADR treatment resulted in the elevation of total and free plasma carnitine, indicating an impaired uptake by cells. The most important carrier in carnitine uptake is the organic cation transporter OCTN2. Physiologically OCTN2 is involved in the intestinal absorption and renal reabsorption of L-carnitine and also plays a major role in tissue distribution. Variations in transport rates and in levels of expressed mRNA are also found in different tissues (Lamhonwah et al. 2003; Ramsay et al. 2001; Xuan et al. 2003).

White blood cells (WBCs) are known to reflect changes in FA metabolism which are known to be associated with physiologic alterations (Choi et al. 2003; Gardner et al. 2005; Karlic et al. 2003; Nakashima et al. 2004; Zeibig et al. 2005). A correlation of simultaneous differential gene expression profiles in the WBCs and heart following anthracycline-induced cardiomyopathy has been shown to exist in the rat, thus suggesting that blood cells may serve as a surrogate marker for the heart. Treatment resulted in the downregulation of a number of genes, including the carnitine transporter OCTN2 both in the WBCs and heart (Brown et al. 2002). We therefore estimated the relative mRNA amounts of carnitine acyltransferases in the WBCs of the patients participating in this study.

Materials and methods

Experimental approach

Forty patients with non-Hodgkin lymphomas (NHLs) (three CLL, two MALT-lymphomas, three mantle cell lymphomas, 16 centroblastic centrocytic lymphomas, 16 high malignant NHL) of a median age of 64 years (placebo group) and 66 years (carnitine group) were randomly assigned to the L-carnitine or to the unsupplemented group ($n = 20$ for each group). The subjects were informed of the possible risks involved in this study. All subjects gave their informed consent before participation. The study was approved by the Ethics Commission of Hanusch Hospital.

Quality of life was assessed using a standardized questionnaire (Hofmann et al. 1993; Tuchler et al. 1992). A stratification based on impairment of cardiac function (ICF) was made.

Of the six patients with ICF, three were assigned to the carnitine and three to the placebo group.

Patients received an infusion containing 3 g L-carnitine (L-CARNITIN "Fresenius" infusion solution) before each chemotherapy cycle, followed by oral administration of 1 g L-carnitine (L-CARNITIN "Fresenius", drinking solution)/day during the following 21 days.

Chemotherapy consisted of six so-called CHOP cycles (day 1: cyclophosphamide 750 mg/m^2 in 500 ml NaCl, vincristine 1.4 mg/m^2, max. 2 mg absolute; doxorubicine 50 mg/m^2 in 259 ml NaCl; days 2–5: prednisolone 100 mg p.o.). Cumulative doxorubicine doses of up to 600 mg/m^2 were thus reached in this study.

Fasting morning blood samples were taken before the first chemotherapy, after the third and sixth CHOP cycle (=4 months after starting the first chemotherapy) and in the washout phase, 2 months after the last chemotherapy.

The healthy control group consisted of 51 elderly persons (49 females, 3 males) with normal hematological parameters. They were residents of two geriatric units. The mean age of the elderly females was 87.5 years (range 78–95 years, median 87 years), that of the elderly males 88.3 years (range 82–93 years, median 90 years). Ten of them (all females) received L-carnitine (2 g carnitine tartrate/day) as a nutritional supplement for 3 months. Blood samples were taken during routine health checkups after informed consent.

Blood samples

Venous blood samples were collected in plain evacuated tubes from a forearm vein with minimal stasis after approximately 10 min of rest in a sitting position. After separation of cell-free plasma for L-carnitine determination and analysis of the serum lipid profile the mononuclear cells (WBCs) were enriched by density gradient centrifugation using a Ficoll–Hypaque separation medium (density according to fraction index: 1.077) and washed several times in phosphate buffered saline. Aliquots of 1–10 million mononuclear blood cells were frozen in 4 M GTC (guanidine isothiocyanate) for preparation of mRNA and cDNA for subsequent reverse transcriptase polymerase chain reaction (RT-PCR).

Analysis of mRNA expression

All mRNA analyses were made from at least one million WBCs collected in EDTA tubes. Isolation of mRNA and preparation of cDNA was carried out applying

standard procedures and using commercially available kits. Quantitative RT-PCR assay was carried out using the LightCycler™ System (Roche). Dilutions of 200–2 ng of cDNA were used in each assay.

For the quantification of CPT1A, CPT1B, CRAT, microsomal CPT and OCTN2 dilutions of known PCR products or cloned amplimers (ranging from 1 ng to 10 fg) were included as standards in each assay for quantitative PCR.

Relative mRNA levels were evaluated by calculating the number of copies of the analyzed gene per 100 copies of beta-actin, which was expressed at the most constant levels per amount of cDNA (1,000 copies/ng c-DNA; SD ±10) before mobilization and after apheresis. We thus used beta-actin as an internal standard. In order to facilitate comparison, results were calculated as a percentage of the values for normal WBCs as previously determined. Further details of primers and reaction conditions are shown in Table 1.

Analysis of plasma carnitine

Perchloric acid extracts of plasma were used for assaying free carnitine and short-chain acylcarnitine. The carnitine esters were saponified and assayed as free carnitine by a radioenzymatic method (Lohninger et al. 1990), with two modifications: HEPES instead of Tris buffer, and *N*-ethylmaleimide instead of tetrathionate.

Analysis of plasma lipids

Free fatty acids, free cholesterol, cholesteryl esters and triglycerides were determined directly from total lipid extract by capillary gas chromatography as described previously. For FFA determination the programmed temperature vaporizer (PTV) injector was heated from 40°C (sample introduction) to 190°C. In a second gas chromatographic run the PTV injector was heated from 60°C (sample introduction) to 400°C to enable determination of the other lipid classes, except phospholipids (Lohninger et al. 1990).

Statistics

Medians and quartiles were calculated from all data. Differences between unpaired data were tested for significance by the Mann–Whitney *U*-test, between paired data by the Wilcoxon matched-pairs signed rank test.

As coefficient of correlation Kendall's tau together with the corresponding significance test was calculated. A *P*-value of 0.05 (two-sided) was chosen as level of significance. No alpha-correction was applied.

Results

Clinical parameters in general

As regards quality of life and quality of health no significant differences were reported at any point of time. However, a better quality of life was reported in both groups after therapy than at commencement of treatment. Survival time was not significantly longer in the carnitine group, duration of remission was similar in both the carnitine and placebo group.

Cardiac parameters

Echocardiographic parameters were checked before the first, after the third, before the fourth and after the sixth chemotherapies. At no point of time were any significant differences observed in the size of the left ventricle (LVIDD), systolic function (EF) and E/A ratio between the placebo and carnitine group (Table 2).

Table 1 Primers and PCR conditions

PCR primer	Sequence	PCR product size (bp)	Annealing temp. (°C)/time (s)	Extension temp. (°C)/time (s)	Acquisition temp. (°C)/time (s)
β-Actin	S: 5′-TgccATccTAAAAgccAc-3′ A: 5′-TcAAcTggTcTcAAgTcAgTg-3′	289	64/5	72/34	83/1
CPT1A	S: 5′-ccTTccAAcTcATTcAg-3′ A: 5′-ccAggATccTcTgcATcTg-3′	298	62/5	72/34	87/1
CPT1B	S: 5′-ggTgAAcAgcAAcTATTATgTc-3′ A: 5′-ATccTcTggAAgTgcATc-3′	348	62/6	72/34	87/1
CPT 2	S: 5′-gggAAgggAAgggAgAcgAg-3′ A: 5′-ccAAgAcAcTgcgTcAggAc-3′	173	63/5	72/34	92/1
CRAT	S: 5′-gAAgcccTTcTccTT-3′ R: 5′-cTccccTAcAccTccTgAg-3′	175	64/5	72/34	91/1
mCPT	S: 5′-cccTcAcATgAcAgAAgAc-3′ A: 5′-cTccTgcATgAcAAAcTTc-3′	270	64/5	72/34	83/1
OCTN2	S: 5′-TccAAgTcAcAcAAggATg-3′ A: 5′-TcccTAgAggAAggTggTg-3′	246	62/5	72/34	86/1

Table 2 Cardiac parameters before (pre) and after (post) therapy and in the washout phase (WP)

Parameter	Carnitine group ($n=20$)			Control group ($n=20$)		
	Pre	Post	WP	Pre	Post	WP
LVIDD (mm)	50.2 (4.1)	49.9 (5.0)	52.0 (5.1)	51.7 (4.1)	51.4 (5.6)	51.4 (5.6)
EF (%)	61.0 (6.0)	58.3 (5.9)	59.4 (7.4)	61.9 (6.3)	60.0 (6.2)	60.3 (6.5)
E/A ratio[a]	0.8 (0.3)	0.8 (0.5)	0.9 (0.3)	0.9 (0.3)	0.8 (0.2)	0.9 (0.2)

Values in parentheses are standard deviations

[a]E/A ratio describes echocardiographic phases of ventricular filling (cm/s). These have been termed early (E) and late, or atrial (A). The latter phase is of course dependent upon atrial contraction and is therefore absent in atrial fibrillation. Characteristically, when diastolic dysfunction is present, a larger percentage of the end-diastolic volume is the result of late rather than early filling. The E/A ratio is thus reduced in diastolic dysfunction

In addition, there were no changes of single parameters during therapy. It can therefore be assumed that no cardiac problems had arisen or that these could not be proved using the above mentioned parameters.

Influence of L-carnitine supplementation on mRNA levels of carnitine acyltransferases

The results of relative mRNA levels of carnitine acyltransferases and OCTN2 in WBCs were evaluated after unblinding of the study. Figure 1 shows the relative mRNA levels of carnitine acyltransferases and OCTN2 pre-therapy, immediately after the last chemotherapy (4 months after commencement of therapy) and 2 months later, in the washout phase.

CPT1A was significantly ($P=0.001$) stimulated following therapy (at time point 2) in the carnitine group (Fig. 1b). The relative mRNA content of CPT1A was then notably higher in the carnitine group than in the placebo group ($P=0.1$). After L-carnitine supplementation there was a significant correlation between the relative mRNA level of CPT1A and OCTN2 ($P=0.04$; tau = 0.27).

In our control groups of healthy elderly probands the subgroup which received carnitine supplementation had a significantly higher CPT1A mRNA level than the unsupplemented probands (63% higher as compared with the control group).

Rise of carnitine transporter OCTN2 and carnitine acyltransferases in the washout phase

Increased mRNA levels 2 months after the last therapy (in the washout phase) were observed for CPT1A in the placebo group and for CPT1B, OCTN2 and CPT2 both in the placebo group and in the carnitine group, but there were no significant differences or correlations with other genes. This confirmed our observations from the control group of healthy elderly people indicating the relationship between serum carnitine levels and the mRNA levels of OCTN2 and the carnitine acyltransferases CPT1B, CRAT and CPT2.

Carnitine level	OCTN2-mRNA level	mRNA level of CPT1B, CRAT and CPT2
high	high	high
low	high	middle
high	low	middle
low	low	low

Rise of plasma carnitine levels and serum triglycerides in the carnitine group

Figure 1c, d shows the plasma carnitine levels and levels of triglycerides CTGL50 and CTGL54 before and after therapy. Plasma carnitine levels were determined three times, namely (1) before the first chemotherapy, (2) after the sixth CHOP cycle (4 months after commencement of the first chemotherapy) and (3) in the washout phase, 2 months after the last chemotherapy. Following carnitine therapy at time point (2), levels of free carnitine (FC) and total carnitine (GC) in plasma were significantly higher in the carnitine than in the placebo group ($P<0.001$). After therapy levels of short chain acyl carnitines (KA) were 10.7 µg/ml in the placebo and 18.3 µmol/l in the carnitine group, but this difference was not significant ($P=0.09$). After the sixth chemotherapy the level of FC was 36.1 µmol/l in the placebo and 53.4 µmol/l in the carnitine group, and the level of GC 46.9 µmol/l in the placebo and 71.7 µmol/l in the carnitine group. In the washout phase, there were no significant differences in serum carnitine levels between the placebo and the carnitine group. Following therapy serum-triglyceride levels were significantly higher in the carnitine as compared to the placebo group (165.6 vs 240.1 mg/dl).

After therapy triglycerides from the groups CTGL50, CTGL 52 and CTGL54 were significantly higher in the carnitine than in the placebo group. Post-therapy differences between the carnitine and placebo group were 24.2 vs 36.3 mg/ml ($P=0.03$) for CTGL50, 55.1 vs 76.1 mg/ml ($P=0.03$) for CTGL 52 and 17.8 vs 24.5 mg/ml ($P=0.05$) for CTGL54.

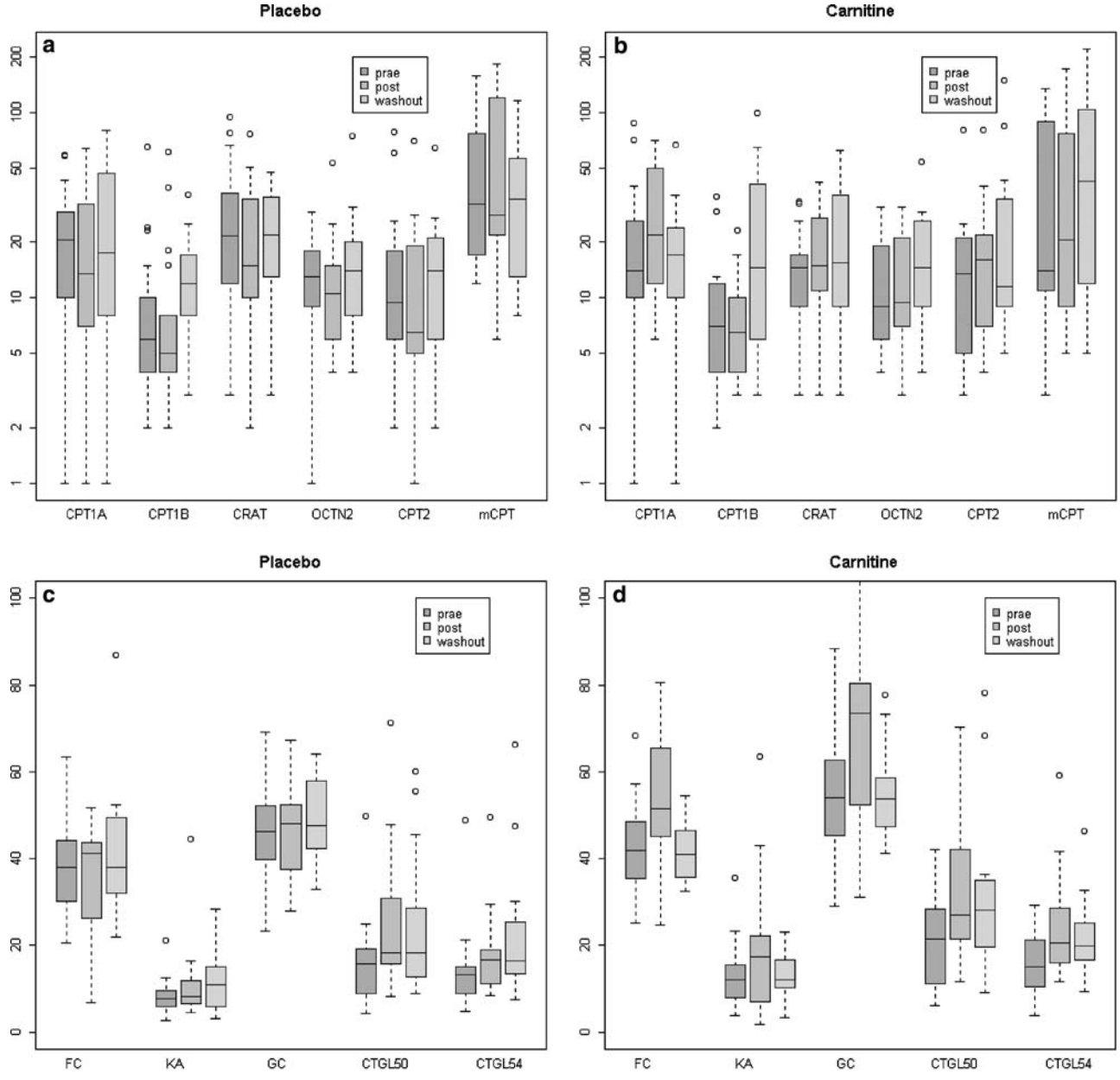

Fig. 1 Relative mRNA levels (normal WBC = 100%) of carnitine acyltransferases (CPT1A, CPT1B, CRAT and CPT2) as well as carnitine transporter OCTN2 are shown for the placebo group (**a**) and the carnitine group (**b**). Relative mRNA levels of CPT1A was significantly increased in the carnitine group ($P < 0.001$). Serum carnitine levels (*FC* free carnitine, *KA* short chain acyl carnitines, *GC* total carnitine) and two types of triglycerides (CTGL50 and CTGL54) are shown for the placebo group (**c**) and the carnitine group (**d**). Following carnitine therapy at time points (1) and (2), levels of free carnitine (*FC*) and total carnitine (*GC*) in plasma were significantly higher in the carnitine group as compared to the placebo group ($P < 0.001$). After therapy, triglycerides, from groups CTGL50and CTGL54, were significantly higher in the carnitine group as compared to the placebo group. Differences between carnitine group and placebo group after therapy were 24.2 vs 36.3 mg/ml ($P = 0.03$) for CTGL50 and 17.8 vs 24.5 mg/ml ($P = 0.05$) for CTGL54. *Box plots* are displaying medians (*middle line*), 25th and 75th percentiles (*boxes*) and upper and lower extremes (*whiskers*). The ° symbols represent outliers

Relationship between free carnitine in plasma and mRNA levels of CPT1A

Figure 1 shows the parallel course of FC values and CPT1A-mRNA levels during the study period. After therapy, a slight (non-significant) decrease of FC and CPT1A was observed in the placebo group (Fig. 1a) whereas an increase of both FC and CPT1A was measured in the carnitine-treated group (Fig. 1b, d). At this point of time both FC and CPT1A were significantly higher in the carnitine than in placebo group. While there was a significant correlation between FC and CPT1A at the first and the second points of time ($P = 0.004$ and $P = 0.06$, respectively), at the third point

of time no significant correlation was observed ($P = 0.3$).

We defined those patients in whom serum carnitine (both KA and GC) and mRNA levels of one or more enzymes had increased by more than 50% as ''responders.'' The group of ''responders'' included all three patients with CLL (two of them belonging to the carnitine group). Of the NHL patients 14.3% (five of 35 patients, four of them from the carnitine group) were ''responders'', but neither of the two MALT (mucosa associated lymphoid tissue) lymphoma cases—one in the carnitine group, one in the placebo group—were included in this group.

The above mentioned ''responders'' included one person with NHL, in whom, in addition to CPT1A, the relative mRNA levels of other carnitine acyltransferases (CPT1B, CRAT, CPT2, microsomal CPT) were also higher. In two NHL cases the mRNA level of microsomal CPT had increased in addition to CPT1A. This underlines the close link between the serum carnitine concentration and mRNA levels of carnitine acyltransferases in WBCs.

Discussion

Anthracyclines (doxorubicin) are constituents of many polychemotherapy regimens as used for the patients with B-cell NHLs who were investigated in the present study. The cytostatic effect results from intercalation, inhibition of topoisomerase II, inhibition of tyrosine kinases, generating reactive oxygen species (Olson and Mushlin 1990; Rabbani et al. 2005; Singal et al. 1997) and induction of apoptosis. There is a known cardiotoxicity for anthracyclines which has been extensively reviewed (Minotti et al. 2004), the cumulative recommended maximum dose being 600 mg/m^2 (Lefrak et al. 1973) in view of the risk for dilatative cardiomyopathy.

In previous studies the protective effect of L-carnitine in anthracycline-induced cardiotoxicity has been demonstrated through histopathologic investigations of myocardial tissue. A remarkable reduction of mitochondrial lesions has been found to occur following inhibition of apoptosis under adjuvant L-carnitine therapy (Abd-Allah et al. 2005; Nakamura et al. 2000; Paterna et al. 1984). The mitochondria supply most of the energy necessary for the function and survival of cells. Consequently patients with cardiomyopathy showed an improved ejection fraction following therapy with L-carnitine (Gurlek et al. 2000).

Although carnitine is also known to be a radical scavenger (Kraemer et al. 2003), it has been suggested that L-carnitine does not simply interfere with oxygen free radical formation (Garcia-Ruiz et al. 1997; Quillet-Mary et al. 1997). It is well established that ADR inhibits both CPT1 and CPT2 activity in a dose-dependent manner (Yoon et al. 2003). In fact, cardiac injuries induced by ischemia (Grynberg 2005) or doxorubicin treatment (McFalls et al. 1986) are known to be accompanied by reduced L-carnitine levels in cardiac myocytes.

Furthermore the reduction of L-carnitine is associated with inhibition of CPT1 in cardiac tissue after adriamycin treatment (Abdel-aleem et al. 1997). In vitro studies have shown that low carnitine levels in the culture media result in the downregulation of CPT1 and CRAT. Carnitine administration restored the mRNA expression in a dose-dependent manner (Lohninger et al. 2002).

The anti-apoptotic factor Bcl-2 is located in the mitochondrial membrane in close association with CPT1 (Paumen et al. 1997a), and L-carnitine is important for the regulation of apoptotic processes both at the mitochondrial level and at the level of ceramide synthesis and signaling (Mutomba et al. 2000; Paumen et al. 1997b). Due to impaired FA oxidation carnitine deficiency is associated with high levels of FFAs. In the present study the levels of FFAs were elevated at the beginning of the study in both the placebo and carnitine group and remained in the upper range throughout the study period. High levels of FFAs are the main cause of insulin resistance, which, in addition to diminished FA utilization, disturb energy production from glucose in heart and skeletal muscle (Kolovou et al. 2005; Mingrone et al. 1999).

Because of pre-existing cardiac problems one patient had to be omitted from this study. In the remaining patients no symptoms of cardiotoxicity were observed, but a higher risk for patients with pre-existing cardiovascular diseases has been reported (Anderlini et al. 1995). In our study we observed no influence of L-carnitine on anthracycline action—e.g., on survival times, relapse rate and major clinical parameters. This confirms previous studies indicating that carnitine treatment reverses the dysfunction of FA metabolism in non-tumorous tissues of cancer patients without affecting anti-cancer therapeutic efficacy (Cruciani et al. 2004; Peluso et al. 2000; Sayed-Ahmed et al. 2000).

The results of this study show that increased plasma carnitine levels following supplementation with L-carnitine induce enzymes of FA metabolism. The initial concentrations of total plasma carnitine (TC) were in general at lower average levels (40–55 µmol/l). After therapy TC values rose above the normal level to 71.7 µmol/l in the carnitine group. Also for FC (53.4 µmol/l) and for short chain acyl carnitine (SA) (18.3 µmol/l) values were above normal levels in the carnitine group. There was a significant rise in plasma carnitine levels in the carnitine group but not in the placebo group. Triglyceride levels were higher in the carnitine group but did not reach the pathologic range.

The effect of L-carnitine on metabolism depends on the intracellular concentration which is itself dependent on the activity of OCTN2. The most sensitive technique for analyzing the effect of L-carnitine on cellular metabolism is by analyzing the mRNA synthesis of carnitine acyltransferases which can be activated in cell culture within a few hours after L-carnitine administration.

Based on experience from our previous studies (Karlic et al. 2003), we analyzed the mRNA expression of carnitine acyltransferases and OCTN2 by quantitative RT-PCR (real time PCR) in WBCs. However, it must be mentioned that the fraction of mononuclear blood cells used in this study does not include several cell types present in whole blood samples: neutrophils, basophils, platelets, reticulocytes and red blood cells.

CPT1A was the only gene which could be stimulated by L-carnitine supplementation both in the patients of this study and in healthy elderly persons who were used as controls. Our data from human WBCs and rat liver (Karlic et al. 2002) were confirmed by recent studies that show an improved activity of mitochondrial enzymes by carnitine supplementation in skeletal muscle and heart (Kumaran et al. 2004, 2005) and brain (Haripriya et al. 2005). Recently, a possible concordant regulation of carnitine associated enzymes after endurance exercise training in WBCs and muscle has been shown (Zeibig et al. 2005).

This is also documented by the correlation between concentration of free carnitine and relative mRNA levels of CPT1A. It appears that free radicals could play a role in OCTN2 expression (Brown et al. 2002), and oxidative stress could be the cause of downregulation of OCTN2 in our patients during therapy. In addition OCTN2 is directly inhibited by a number of xenobiotics, especially lipophilic organic cations, that is, the antibiotic emetine and the ion channel blockers quintine and verapamil (Wagner et al. 2000), zwitterionic compounds such as the β-lactam antibiotics cephaloridine, cefepime, ceftazidime and cefluprenam (Ganapathy et al. 2000). This could be the reason for a higher efficiency of L-carnitine as a nutritional supplement found in healthy probands (Karlic and Lohninger 2004; Karlic et al. 2003).

In conclusion, the results of this study provide evidence that doxorubicin-induced blocking of L-carnitine uptake may be attenuated by L-carnitine treatment. L-Carnitine supplementation can stimulate FA metabolism in WBCs and change the lipid profile of serum. Further studies are necessary to find out whether an additional application of radical scavengers (e.g., bioflavonoids and/or lipoic acid; Al-Majed et al. 2002; Quiles et al. 2002) could improve the cytoprotective effect of L-carnitine by protecting the carnitine transporter OCTN2 from radical damage.

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