ANTICANCER RESEARCH *25*: 2681-2688 (2005)

Acetyl-L-Carnitine Prevents and Reverts Experimental Chronic Neurotoxicity Induced by Oxaliplatin, Without Altering its Antitumor Properties

ORLANDO GHIRARDI[1], PIETRO LO GIUDICE[1], CLAUDIO PISANO[1], MARIO VERTECHY[1], AUGUSTA BELLUCCI[1], LOREDANA VESCI[1], SANTE CUNDARI[1], MARIAROSARIA MILOSO[2], LAURA MARIA RIGAMONTI[2], GABRIELLA NICOLINI[2], CLAUDIO ZANNA[1] and PAOLO CARMINATI[1]

[1]*Research and Development, Sigma-Tau S.p.A. Industrie Farmaceutiche Riunite, Rome;*
[2]*Department of Neuroscience and Biomedical Technologies, University of Milan "Bicocca", Monza, Italy*

Abstract. *Background: Oxaliplatin (OHP) is severely neurotoxic and induces the onset of a disabling sensory peripheral neuropathy. Acetyl-L-carnitine (ALC), a natural compound with neuroprotective action, was tested to determine whether it plays a protective role in OHP-induced neuropathy. Materials and Methods: Peripheral neuropathy was induced in Wistar rats, and the effect of OHP alone or in combination with ALC was assessed, using behavioral and neurophysiological methods. Moreover, ALC interference on OHP antitumor activity was investigated using several in vitro and in vivo models. Results: ALC-co-treatment reduced the neurotoxicity of OHP when it was coadministered. Furthermore, the administration of OHP, once OHP-induced neuropathy was established, significantly mitigated its severity. Finally, experiments in different tumor systems indicated that ALC does not interfere with the antitumor effects of OHP. Conclusion: ALC is effective in the prevention and treatment of chronic OHP-induced peripheral neurotoxicity in an experimental rat model.*

One of the most interesting platinum-derived drugs is oxaliplatin [(trans-l)1,2-diaminocyclohexaneoxalatoplatinum(II)] (OHP), an agent with a 1,2-diaminocyclohexane carrier ligand that produces bulkier DNA conjugates than other platinum-derived drugs due to the restricted freedom of motion of the platinum atom (1). OHP is a very promising antineoplastic drug, despite its neurotoxicity.

Correspondence to: Dr. Orlando Ghirardi, Department of Neuroscience, Research and Development, Sigma-Tau S.p.A. Industrie Farmaceutiche Riunite, Via Pontina, Km 30.400, Pomezia (Rome), Italy. Tel: 39-06-9139.3324, Fax: 39-06-9139.3988, e-mail: orlando.ghirardi@sigma-tau.it

Key Words: Acetyl-L-carnitine, oxaliplatin, neurotoxicity.

Acute neurotoxicity, manifested as paresthesias and dysesthesias in the extremities, triggered or enhanced by exposure to cold, is never dose-limiting. Chronic and cumulative peripheral neurotoxicity caused by OHP, on the contrary, represents a dose-limiting side-effect. Symptoms similar to those seen with cisplatin, depend on the cumulative dose of OHP and occur in most patients at total cumulative doses of >600 mg/m^2 (2, 3). Such neurological toxicity is generally reversible, but it may be severe and last for several months. The features of the chronic peripheral neurotoxicity of platinum-based drugs can be studied using neurophysiological, behavioral, pathological and analytical methods in several well-characterized animal models (4-9).

Acetyl-L-carnitine (ALC) is a member of a family of natural compounds which perform an essential role in intermediary metabolism (10-13). It has been found to be effective as a neuroprotectant in peripheral nervous system disorder models such as diabetic neuropathy (14-16) and motoneuron survival after axotomy (17).

In the present study, the effect of ALC as a putative neuroprotectant capable of preventing the onset of, or enhancing recovery from, OHP-induced peripheral neurotoxicity was studied in rats, using behavioral and neurophysiological methods. Moreover, a lack of interference by ALC with the antitumor activity of OHP in both *in vitro* and *in vivo* models was demonstrated.

Materials and Methods

The animals were housed in a limited access animal facility. The animal room temperature and relative humidity were set at 22±2˚C and 55±10%, respectively. Artificial lighting provided a 24-hour cycle of 12 hours light/12 hours dark (7 a.m. - 7 p.m.). The care and husbandry of animals were in accordance with European Directives no. 86/609, and with Italian D.L. 116, January 27, 1992. All experiments were approved by the Sigma-Tau veterinarian.

Drugs. ALC was supplied by Sigma-Tau (batch 9747). Oxaliplatin was provided by Debiopharm SA (Lausanne, Switzerland).

Behavioral experiment. Seventy-four 15 to 16-week-old (360-380 g) male Wistar rats (Charles River, Calco, Italy) were used. OHP, dissolved in sterile saline, was administered at a dose of 3 mg/kg *i.p.* three times weekly for the first week, twice a week for the second week and once weekly for the third week. Control animals were treated with the vehicle alone. ALC was dissolved in sterile saline and administered at a dose of 100 mg/kg/day *s.c.* The effect of ALC was evaluated both when administered together with OHP and when administered after OHP.

The Randall-Selitto paw-withdrawal test was used to assess mechanical hyperalgesia (18). The nociceptive flexion reflex was quantified with an analgesimeter (Ugo Basile, Varese, Italy), which applies a linearly increasing mechanical force to the dorsum of the rat's hindpaw. The nociceptive threshold was defined as the force which induced rat paw withdrawal. Rats were habituated to the testing procedure during the week prior to the experiments (19). The test was conducted just before the experiment began (basal) and weekly following OHP administration.

Neurophysiological experiment. Ninety-four 11 to 13-week-old (175-225 g) female Wistar rats (Charles River Laboratories, Iffa-Credo, France) were used for this set of experiments. In all the experimental paradigms, neuropathy was induced by repeated *i.v.* OHP administration (3 mg/kg twice a week for 4 weeks). Control animals were treated with the vehicle alone.

In the first experiment, ALC was administered *i.v.* to a group of rats at a dose of 30 mg/kg/ twice a week for 4 weeks, together with OHP.

In the second experiment, ALC was administered at a dose of 150 mg/kg *p.o.* in drinking water starting one week before OHP administration.

In the third experiment, ALC was administered to a group of rats at a dose of 100 mg/kg/day *s.c.*, starting on the same day as the first OHP administration. At the end of the treatment, OHP neuropathic rats were divided into two homogeneous groups: one receiving ALC treatment (100 mg/kg/day *s.c.* for 3 weeks), the other used as a neuropathic control.

Before, during and after the treatment, each animal underwent determination of sensory nerve conduction velocity (SNCV) in the tail (20, 21). Briefly, the antidromic SNCV in the tail nerve was assessed by distally placing recording ring electrodes in the tail, while the stimulating ring electrodes were placed 5 cm and 10 cm proximally, according to the recording point. The latencies of the potentials recorded at the two sites after nerve stimulation were determined (peak-to-peak), and nerve conduction velocity was calculated accordingly. All the neurophysiological determinations were performed under standard conditions. Animal body temperature was maintained constant (37˚C) throughout the experiment (BM 70002-type thermoregulator for animals, Biomedica Mangoni, Pisa, Italy).

A summary of the experimental *in vivo* paradigms is reported in Table I.

Statistical analysis. Statistical comparisons between groups were performed by using repeated measure analysis of variance (ANOVA) followed by the Student-Newman-Keuls (SNK) multiple comparison test. Differences in p-values <0.05 were considered significant.

Table I. *Summary of the experimental paradigms in neuroprotection studies.*

	Drug	Single dose	Schedule	Route
Behavioral study				
	OHP	3 mg/kg	three times/week x 1 week twice/week x 1 week once/week x 1 week	*i.p.*
	ALC	100 mg/kg	daily	*s.c.*
Neurophysiological studies				
	OHP	3 mg/kg in all experiments	twice/week x 4 weeks	*i.v.*
Experiment 1	ALC	30 mg/kg	twice/week x 4 weeks	*i.v.*
Experiment 2	ALC	150 mg/kg	daily (starting on day –7)	*o.s.*
Experiment 3	ALC	100 mg/kg	a) daily during treatment b) daily during follow-up treatment x 3 weeks	*s.c.*

Cytotoxicity experiments

In vitro studies. The NCI-H460 non-small cell lung carcinoma (NSCLC), HT-29 colon adenocarcinoma, PC3 prostate carcinoma, A2780 ovarian carcinoma and HeLa cervix uteri carcinoma cells were grown in RPMI 1640 (Sigma Chemicals Aldrich, Milan, Italy). The IGROV-1 ovarian carcinoma cells were grown in DMEM (Life Technologies, Inc.-Invitrogen, Paisley, UK). The LoVo colon adenocarcinoma was grown in Ham's F12 (Life Technologies). NTERA-2 testicular teratocarcinoma was grown in Vitacell DMEM (American Type Culture Collection, Manassas, VA, USA).

For the cytotoxicity assay, cells were seeded in 96-well tissue culture plates (Corning, New York, NY, USA) and were allowed to attach and recover for at least 24 h. Varying concentrations of OHP alone or combined with ALC (200 μg/mL) corresponding to 1 mM were then added to each well. The plates were incubated for 2 h and then washed before being incubated without drugs for an additional 72 h. The number of surviving cells was then determined by staining with sulforhodamine B (SRB), as described by Skehan *et al.* (22). IC_{50}±SD values were evaluated using an "ALLFIT" program.

In vivo studies. Swiss nu/nu female mice and BDF1 female mice, 5 to 6 weeks of age (Harlan, Correzzana, Italy), were used for this set of experiments.

L1210 murine leukemia was routinely maintained by *i.p.* passages (every 10-14 days) of $1x10^5$ cells/mouse in BDF1 mice. For anti-tumor activity experiments, 5 mL of Hank's 199 medium were injected *i.p.* Cells were withdrawn from donor mice, diluted 1:10 (v/v) in the same medium and re-suspended at a density of $1x10^6$ cells/mL. Then, $1x10^5$ cells/100 μl/mouse were injected *i.p.* into BDF1 mice. Each experimental group included 8 tumor-bearing mice. Tumors were implanted on day 0. OHP was delivered *i.p.* in a volume of 10 mLl/kg at doses of 12.5 and 6.25 mg/kg according to q4dx3 schedule, and ALC was given at 100 mg/kg *p.o.* for 14 days; both drugs were administered starting 1 day after tumor injection. Median survival time for each experimental group was analyzed. Increase in Life Span% (ILS%) was evaluated as [(median survival time of treated group/median survival time of control group) x 100] – 100. Cured mice were evaluated 100 days after tumor implantation.

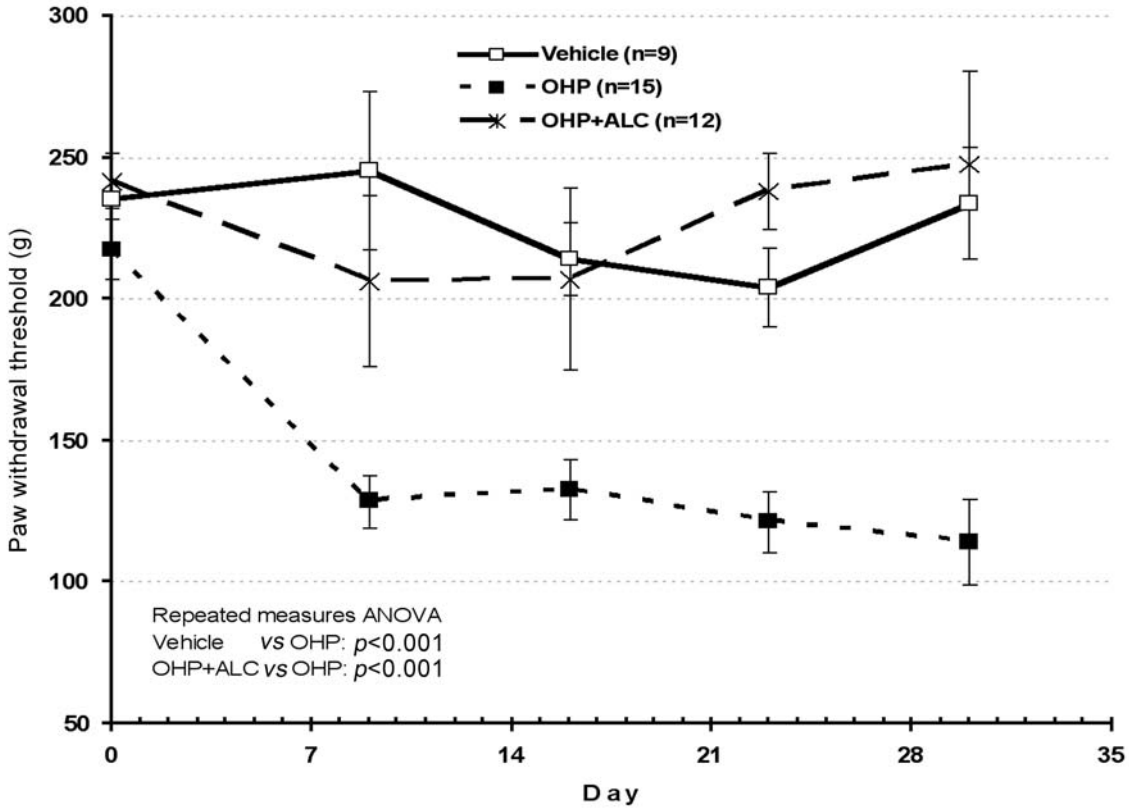

Figure 1. *ALC and OHP-induced neuropathy: mechanical nociceptive threshold score (ALC treatment starting from day 1 of OHP administration).*

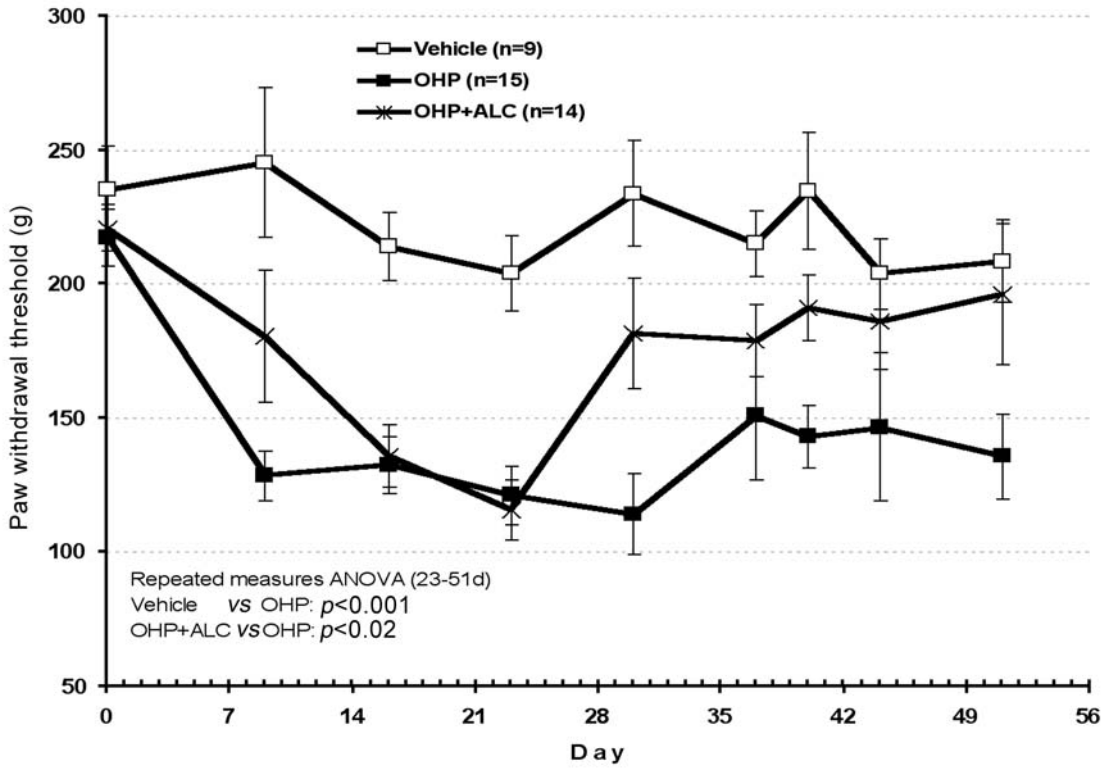

Figure 2. *ALC and OHP-induced neuropathy: mechanical nociceptive threshold score (ALC treatment starting from day 24 of OHP administration).*

Table II. *Intravenous ALC and OHP-induced neuropathy. Sensory nerve conduction velocity (m/s).*

Experiment 1.

Treatment group	Baseline	After treatment
Vehicle (6)	31.00±0.35	40.57±0.43
OHP (8)	30.50±0.27	31.85±0.93[a]
OHP+ALC (8)	30.54±0.69	36.46±0.69[b,c]

Values represent mean±SEM
Repeated measures ANOVA:
OHP *vs.* Vehicle: $F_{(1,12)}= 83.39$; $p<0.0000$
OHP *vs.* OHP+ALC: $F_{(1,12)}= 12.57$; $p<0.005$
OHP+ALC *vs.* Vehicle: $F_{(1,12)}= 13.06$; $p<0.005$
One-way ANOVA followed by SNK test:
[a]$p<0.001$, [b]$p<0.05$ *vs.* Vehicle
[c]$p<0.001$ *vs.* OHP

Table III. *Oral ALC and OHP-induced neuropathy. Sensory nerve conduction velocity (m/s).*

Experiment 2.

Treatment group	Baseline	After treatment
Vehicle (8)	33.05±0.49	37.11±0.60
OHP (12)	32.36±0.59	31.41±0.58[a]
OHP-ALC (11)	33.32±0.55	35.80±0.66[b]

Values represent mean±SEM
Repeated measures ANOVA:
OHP *vs.* Vehicle: $F_{(1,18)}= 27.47$; $p<0.0001$
OHP *vs.* OHP-ALC: $F_{(1,21)}= 12.28$; $p<0.005$
OHP-ALC *vs.* Vehicle: $F_{(1,17)}= 1.60$; n.s.
One-way ANOVA followed by SNK test:
[a]$p<0.001$ *vs.* Vehicle
[b]$p<0.001$ *vs.* OHP

HT-29 human colon adenocarcinoma cells from *in vitro* cell cultures were injected *s.c.* using $5x10^6$ cells/mouse into the right flank of Swiss nude mice. Each experimental group included 8 tumor-bearing mice. Animals received two *i.p.* doses (15 and 10 mg/kg) of OHP during the fourth, sixth and tenth day after tumor injection. ALC was administered *p.o.* for 14 days starting 1 day after tumor implantation. Drug efficacy was assessed as tumor volume inhibition (TVI) in drug-treated *versus* control mice. TVI percentage was evaluated after final treatments as 100– (mean TVI-treated/mean TV control x 100).

The toxic effects of drug treatment were assessed as described below: a) body weight loss (BWL) was calculated as follows: BWL (%) = 100–(mean body weight days x/mean body weight day 1 x 100), where day 1 is the first day of treatment, and day x is any day thereafter. The highest (maximum) BWL is reported in the Tables. Mice were weighed twice a week throughout the experimental period; b) lethal toxicity was defined as any death in treated groups occurring before any control death. Mice were inspected daily for mortality.

Statistical analysis. The *in vivo* experimental results were compared using the Mann-Whitney test. Differences were considered significant at $p<0.05$.

Results

No side-effects were associated with the administration of ALC *i.v.*, *s.c.* or *p.o.*

Behavioral experiment: subcutaneous ALC prevents OHP-induced hyperalgesia and accelerates recovery after onset. The administration of OHP alone induced a significant, early and long-lasting lowering of the mechanical nociceptive threshold compared to the control group (Figures 1 and 2).

As depicted in Figure 1, it can be seen that ALC prevented a lowering of the mechanical nociceptive threshold induced by OHP (vehicle *vs.* OHP, $p<0.001$) when the 2 drugs were co-administered (OHP+ALC *vs.* OHP, $p<0.001$. The effect of repeated ALC treatments, when ALC administration was initiated 24 days from the first OHP administration, is illustrated in Figure 2.

ALC treatment was able to restore the mechanical nociceptive threshold within a few days ($p<0.02$ *vs.* OHP group from days 23 to 51 from the first OHP administration). The paw-withdrawal threshold values obtained in the OHP+ALC group were not significantly different from those observed in the vehicle-treated animals at the end of the observation period, while the values obtained in the OHP group were significantly lower.

Neurophysiological experiments: intravenous, subcutaneous and oral ALC administration prevents OHP-induced SNCV impairment. In the 3 experiments, OHP induced a significant reduction in SNCV with respect to vehicle- treated animals.

In the first experiment, reduction in SNCV was counteracted by co-administration of ALC *i.v.* (Table II) at the determination performed after treatment.

Similarly, OHP-induced SNCV reduction was counteracted by the contemporary administration of ALC, which was started 1 week before the first OHP administration (Table III).

In the third experiment, ALC was administered to a group of rats at a dose of 100 mg/kg/day *s.c.*, starting on the same day as the first OHP administration. In this study as well, SNCV reduction was counteracted by contemporary treatment with ALC (Table IV).

Neurophysiological experiments: subcutaneous ALC enhances the recovery of OHP-induced SNCV impairment. In the third experiment, after the treatment period, neuropathic rats were divided into two homogeneous groups: one receiving ALC

Table IV. *Subcutaneous ALC and OHP-induced neuropathy. Sensory nerve conduction velocity (m/s) in treatment period.*

Experiment 3.

Treatment group	Baseline	After treatment
Vehicle (6)	30.98±0.58	35.80±0.68
OHP (26)	31.31±0.34	30.69±0.22[a]
OHP-ALC (9)	31.44±0.50	33.63±0.85[b,c]

Values represent mean±SEM
Repeated measures ANOVA:
OHP *vs.* Vehicle: $F_{(1,30)}=27.76$; $p<0.0001$
OHP *vs.* OHP-ALC: $F_{(1,33)}= 12.67$; $p<0.005$
OHP-ALC *vs.* Vehicle: $F_{(1,13)}= 1.54$; n.s.
One-way ANOVA followed by SNK test:
[a]$p<0.001$, [b]$p<0.05$ *vs.* Vehicle
[c]$p<0.001$ *vs.* OHP

Table V. *Subcutaneous ALC and OHP-induced neuropathy. Sensory nerve conduction velocity (m/s) in the follow-up period.*

Experiment 3.

Treatment group	After treatment	Follow-up (weeks)		
		1	2	3
Vehicle (6)	35.80±0.68	37.77±0.93	38.18±0.60	39.33±0.3
OHP (13)	30.76±0.20[a]	31.59±0.96[a]	32.01±0.66[a]	35.90±0.63[a]
OHP-ALC (13)	30.62±0.41[a]	34.11±0.72[b,c]	35.06±0.53[c,d]	36.25±0.55[c]

Values represent mean±SEM
Repeated measures ANOVA:
OHP *vs.* Vehicle: $F_{(1,12)}=47.96$; $p<0.0001$
OHP *vs.* OHP-ALC: $F_{(1,19)}= 5.92$; $p<0.05$
OHP-ALC *vs.* Vehicle: $F_{(1,17)}= 25.66$; $p<0.0001$
One-way ANOVA followed by SNK test:
[a]$p<0.001$, [c]$p<0.05$ *vs.* Vehicle
[b]$p<0.01$ [d]$p<0.05$ *vs.* OHP

(100 mg/kg/day *s.c.* for 3 weeks), and the other used as a neuropathic control.

ALC administered at the end of OHP treatment was able to enhance the recovery of SNCV impairment induced by OHP: in fact, during the first 2 weeks of follow-up, SNCV values were significantly higher in the ALC+OHP-treated rats compared to the group treated with OHP alone (Table V).

Cytotoxicity experiments

In vitro studies. The antiproliferative activity of OHP alone and in combination with a high concentration of ALC was also evaluated in a panel of human tumor cell lines (Table VI). In all the cell systems tested, OHP showed marked cytotoxic efficacy. The most sensitive cell lines were the human cell lines IGROV-1, A2780 ovarian, NTERA-2 testicular carcinomas, NCI-H460 non-small cell lung carcinoma, HT-29 and LoVo colon carcinoma, with IC_{50}s in a range between 2.8 and 9.3 μg/mL, followed by HeLa cervix uteri and PC3 prostate carcinoma (IC_{50}s of 16.8 and 58.7 μg/mL, respectively).

However, ALC alone was not cytotoxic for the cells (IC_{50}>200 μg/mL). Moreover, the same concentration of ALC added to cells together with OHP did not influence its growth-inhibitory effect on any human tumor cell lines tested (Table VI).

In vivo studies. In L1210 leukemia-bearing mice, OHP at the approximate maximum tolerated dose of 12.5 mg/kg *i.p.,* given according to the q4dx3 schedule, showed significant antitumor activity with treatment starting 1 day after tumor injection, as it prolonged the lifespan by 354% ($p<0.05$) with 75% LTS (long-term survivors). At 6.25 mg/kg, *i.p.* a potent effect was obtained again with an ILS of 204% ($p<0.001$) and 50% LTS (Table VII).

Table VI. *Cytotoxicity of OHP alone and in combination with ALC on different human tumor cell lines.*

Tumor cell line	IC_{50} (μg/mL)±SD		
	ALC	OHP	OHP+ALC
Ovarian carcinoma			
IGROV-1	>200	2.8±0.4	2.0±0.2
A2780	>200	6.6±2.0	6.7±0.4
Testicular teratocarcinoma			
NTERA-2	>200	6.6±0.4	5.3±0.5
NSCLC			
NCI-H460	>200	9.2±0.5	11.1±2.5
Colon adenocarcinoma			
HT-29	>200	9.7±0.9	10.6±1.2
LoVo	>200	9.3±1.7	11.2±0.8
Cervix uteri carcinoma			
HeLa	>200	16.8±0.1	15.0±0.4
Prostate carcinoma			
PC3	>200	58.7±3.0	56.4±1.2

ALC (100 mg/kg *p.o.*) administered alone daily for 14 days had no antitumor activity, and a combination of ALC with OHP did not influence the antitumor effects of OHP.

The antitumor activity of OHP was also studied in a human tumor xenograft, a colon adenocarcinoma (HT-29). Three *i.p.* injections of OHP at 15 mg/kg on the fourth, sixth and tenth day after tumor implantation induced a significant reduction in tumor weight (TVI=48%, $p<0.01$), but were associated with 8 deaths due to toxicity after the last treatment. At a lower dose of 10 mg/kg, OHP showed a significant antitumor activity (TVI=38%, $p<0.01$) without any notable toxicity. ALC (100

Table VII. *Effect of OHP in combination with ALC on median survival time in BDF1 female mice bearing transplantable L1210 murine leukemia.*

Treatment	Dose (mg/kg)	BWL%[a] max	MST[b] (range of days)	ILS%	LTS[d] day 100
[c]Controls	---	2	22 (18-27)	---	0/8
ALC	100	0	20 (18-28)	0	0/8
OHP	12.5	11	100 (16-100)	*>354	6/8
ALC +OHP	100 12.5	16	100 (29-100)	***>354	5/8
OHP	6.25	2	67 (32-100)	***>204	4/8
ALC +OHP	100 6.25	4	70 (35-100)	***>218	4/8

[a]BWL%=maximum body weight loss during experimental period
[b]MST=median survival time (days)
[c]Controls, vehicle-treated group
Number of long-term survivors on day 100 over the number of mice per group.
***$p<0.001$ and *$p<0.05$ *vs.* vehicle (Mann-Whitney)

Table VIII. *Effect of OHP in combination with ALC on antitumor activity in Swiss nu/nu female mice bearing HT-29 colon carcinoma.*

Treatment	Dose (mg/kg)	Lethality	BWL%[a] max	TVI±SEM[b]	
				+11 days	+18 days
[c]Controls	---	0/8	0	---	---
ALC	100	0/8	0	11	12
OHP	10	0/8	0	**38±5	*40±3
ALC +OHP	100 10	0/8	8	**37±3	*33±2

[a]BWL%=maximum body weight loss during experimental period
[b]TVI=tumor volume inhibition
[c]Controls, vehicle-treated group
**$p<0.01$ and *$p<0.05$ *vs.* vehicle (Mann-Whitney)

mg/kg *p.o.*) given daily for 14 days starting 1 day after tumor implantation was inactive against this tumor histotype. Again, a combination of ALC with OHP did not show any difference in tumor growth compared with OHP alone (Table VIII).

Discussion

The prevention of chemotherapy-induced neuropathy associated with the clinical use of platinum-derived drugs such as OHP is a major goal in clinical oncology. Pre-clinical *in vitro* studies can offer important clues for identifying effective neuroprotective strategies since target side-effects can be tested with different modalities. The peripheral neuropathy induced in rats by repeated administration of OHP has been studied using neurophysiological, pathological, analytical and biochemical methods (9), and it can be safely stated that it is qualitatively similar to that observed in humans, as characterized by the involvement of the sensory nerve fibers secondary to DRG neuronopathy.

In this study behavioral and neurophysiological methods were employed to investigate the possibility that ALC may be able to reduce OHP-induced peripheral neurotoxicity in a chronic *i.v.* administration rat model.

The main action of ALC, as identified to date, is on intermediary metabolism (10-13). Furthermore, different studies have demonstrated a neuroprotective role for this member of the carnitine family in both the central and peripheral nervous systems. Exogenous administration of ALC reduces the severity of experimental diabetic neuropathy (14-16), enhances motoneuron survival after axotomy (17) and modulates control of NGF level in the CNS of adult rats following total fimbria-fornix transection (23) and the rate of transcription of gene coding for the $p75^{NGFR}$ in the basal forebrain and cerebellum of aged rats (24).

Regarding chemotherapy-induced peripheral neurotoxicity, it has already been reported that ALC exerts a protective effect against cisplatin- and paclitaxel-induced neurological impairment in rat models. Moreover, it is noteworthy that the protective effect of ALC was obtained without interfering with the cisplatin or paclitaxel antineoplastic effect *in vitro* or *in vivo*, as evaluated on several solid tumor types. In the present study, it was also confirmed that the OHP antitumor effect is not reduced by ALC-co-treatment in a panel of human cell lines and in *in vivo* murine models. The explanation for this different sensitivity to ALC protection in tumor cells and in normal tissues is still only speculative; it is possible, however, that some of the mechanisms and biochemical pathways modulated by this agent may have tissue-specific relevance.

In the present study, ALC was effective in the prevention of OHP neurotoxicity in different experimental paradigms when the neurological impairment was assessed using both behavioral and neurophysiological methods. Interestingly, ALC administration was also able to enhance the reversal from neurological damage in the follow-up period subsequent to OHP withdrawal.

Given the marked similarities between cisplatin and OHP chronic neurotoxicity, our positive results are not surprising, and it is likely that the mechanism of ALC neuroprotection might be the same, although this has not yet been completely clarified.

The pleiotropicity of effects associated with ALC cannot be explained solely on the basis of the above-mentioned effects on intermediary metabolism. Over the last few years, the possibility that ALC may also be involved in nuclear events has emerged. Moreover, the molecular properties of ALC have led

to the suggestion that it may play a role in histone acetylation and, consequently, in facilitating gene expression (25-27).

In conclusion, our experimental results demonstrate that ALC is effective in the prevention and treatment of chronic OHP-induced peripheral neurotoxicity, thus giving further support to the possible role of ALC as a neuroprotectant in chemotherapy schedules.

Acknowledgements

We thank Patrizia Tobia, Angelo Marconi and Silvio Zavatto for their excellent technical assistance.

References

1 Tashiro T, Kawada Y, Sakurai Y and Kidani Y: Antitumor activity of a new platinum complex, oxalato (trans-l-1,2-diaminocyclohexane)platinum (II): new experimental data. Biomed Pharmacother *43*: 251-260, 1989.

2 Giacchetti S, Perpoint B, Zidani R, Le Bail N, Faggiuolo R, Focan C, Chollet P, Llory JF, Letourneau Y, Coudert B, Bertheaut-Cviktovic F, Larregain-Fournier D, Le Rol A, Walter S, Adam R, Misset JL and Levi F: Phase III multicenter randomized trial of oxaliplatin added to chronomodulated fluorouracil-leucovorin as first-line treatment of metastatic colorectal cancer. J Clin Oncol *18*: 136-147, 2000.

3 Machover D, Diaz-Rubio E, de Gramont A, Schilf A, Gastiaburu JJ, Brienza S, Itzaki M, Merger G, N'Daw D, Vigoud J, Abad A, Francois E, Gamelin E, Marty M, Sastre J, Seitz JF and Ychou M: Two consecutive phase II studies of oxaliplatin (L-OHP) for treatment of patients with advanced colorectal carcinoma who were resistant to previous treatment with fluoropyrimidines. Ann Oncol *7*: 95-98, 1996.

4 Cavaletti G, Tredici G, Marmiroli P, Petruccioli MG, Barajon I and Fabbrica D: Morphometric study of the sensory neurones and peripheral nerve changes induced by chronic cisplatin (DDP) administration. Acta Neuropathol *84*: 364-371, 1992.

5 Cavaletti G, Minoia C, Schieppati M and Tredici G: Protective effects of glutathione on cisplatin neurotoxicity in rats. Int J Rad Oncol Biol Phys *29*: 771-776, 1994.

6 McKeage MJ, Boxall FE, Jones M and Harrap KR: Lack of neurotoxicity of oral bisacetatoammine-dichlorocyclohexylamine-platinum(IV) in comparison to cisplatin and tetraplatin in the rat. Cancer Res *54*: 629-631, 1994.

7 Holmes J, Stanko J, Varchenko M, Ding H, Madden VJ, Bagnell CR, Wyrick SD and Chaney SG: Comparative neurotoxicity of oxaliplatin, cisplatin, and ormaplatin in a Wistar rat model. Toxicol Sci *46*: 342-351, 1998.

8 Cavaletti G, Fabbrica D, Minoia C, Frattola L and Tredici G: Carboplatin toxic effects on the peripheral nervous system of the rat. Ann Oncol *9*: 443-447, 1998.

9 Cavaletti G, Tredici G, Petruccioli MG, Dondè E, Tredici P, Marmiroli P, Minoia C, Ronchi A, Bayssas M and Griffon-Etienne G: Effect of different schedules of oxaliplatin treatment on the peripheral nervous system of the rat. Eur J Cancer *37*: 2457-2463, 2001.

10 Fritz IB: Carnitine and its role in fatty acid metabolism. Adv Lipid Res *1*: 285-334, 1963.

11 Bremer J: Carnitine metabolism and functions. Physiol Rev *63*: 1420-1480, 1983.

12 Stanley CA: New genetic defects in mitochondrial fatty acid oxidation and carnitine deficiency. Adv Pediatr *34*: 59-88, 1987.

13 Bieber LL: Carnitine. Annu Rev Biochem *57*: 261-283, 1988.

14 Lowitt S, Malone JI, Salem AF, Korthals J and Benford S: Acetyl-L-carnitine corrects the altered peripheral nerve function of experimental diabetes. Metabolism *44*: 677-680, 1995.

15 Di Giulio AM, Lesma E and Gorio A: Diabetic neuropathy in the rat: 1. ALC augments the reduced levels and axoplasmic transport of substance P. J Neurosci Res *40*: 414-419, 1995.

16 Kano M, Kawakami T, Hori H, Hashimoto Y, Tao Y, Ishikawa Y and Takenaka T: Effects of ALC on the fast axoplasmic transport in cultured sensory neurons of streptozotocin-induced diabetic rats. Neurosci Res *33*: 207-213, 1999.

17 Fernandez E, Pallini R, Tamburrini G, Lauretti L, Tancredi A and La Marca F: Effects of levo-acetylcarnitine on second motoneuron survival after axotomy. Neurol Res *17*: 373-376, 1995.

18 Aley KO, Reichling DB and Levin JD: Vincristine hyperalgesia in the rat: a model of painful vincristine neuropathy in humans. Neurosci *73*: 259-265, 1996.

19 Taiwo YO, Coderre TJ and Levine JD: The contribution of training to sensitivity in the nociceptive paw-withdrawal test. Brain Res *487*: 148-151, 1989.

20 Cavaletti, G, Minoia C, Schieppati M and Tredici G: Protective effects of glutathione on cisplatin neurotoxicity in rats. Int J Rad Oncol Biol Phys *29*: 771-776, 1994.

21 Tredici G, Cavaletti G, Petruccioli MG, Fabbrica D, Tedeschi M and Venturino P: Low-dose glutathione administration in the prevention of cisplatin-induced peripheral neuropathy in rats. Neurotoxicology *15*: 701-704, 1994.

22 Skehan P, Stoneng R, Scudiero D, Monks A, Mc Mahon J, Vistica D, Warren JT, Bokesch H, Kennedy S and Booyd MR: New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst *82*: 1107-1112, 1990.

23 Piovesan P, Pacifici L, Taglialatela G, Ramacci MT and Angelucci L: Acetyl-L-carnitine treatment increases choline acetyltransferase activity and NGF levels in the CNS of adult rats following total fimbria-fornix transection. Brain Res *633*: 77-82, 1994.

24 Foreman PJ, Perez-Polo JR, Angelucci L, Ramacci MT and Taglialatela G: Effect of acetyl-L-carnitine treatment and stress exposure on the nerve growth factor receptor (p75NGFR) mRNA level in the central nervous system of aged rats. Prog Neuropsychopharmacol Biol Psychiatry *19*: 117-133, 1995.

25 Fanti L, Berloco M and Pimpinelli S: Carnitine suppression of position-effect variegation in *Drosophila melanogaster.* Mol Gen Genet *244*: 588-595, 1994.

26 Pomponi MG and Neri G: Butyrate and acetyl-l-carnitine inhibit the cytogenetic expression of the fragile X *in vitro*. Am J Med Gen *51*: 447-450, 1994.

27 Pisano C, Pratesi G, Laccabue D, Zunino F, Lo Giudice P, Bellucci A, Pacifici L, Camerini B, Vesci L, Castorina M, Cicuzza S, Tredici G, Marmiroli P, Nicolini G, Galbiati S, Calvani M, Carminati P and Cavaletti G: Paclitaxel and cisplatin-induced neurotoxicity: a protective role of acetyl-L-carnitine. Clin Cancer Res *9*: 5756-5767, 2003.

Received December 3, 2004
Accepted April 13, 2005