Chemotherapy-induced Allodinia: Neuroprotective Effect of Acetyl-L-carnitine

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Abstract. Background: We tested the hypothesis that acetyl-L-carnitine (ALC) may have a protective and a curative role in chemotherapy-induced hyperalgesia in vivo, in animal models of cisplatin-, paclitaxel- and vincristine-induced neuropathy. In addition, the possible interaction between ALC and vincristine antineoplastic action was assessed. Materials and Methods: Chemotherapy-induced peripheral neuropathy (CIPN) was induced in different groups of rats. The effect of ALC was evaluated both when its administration was started together with the administration of anticancer drugs ("preventive" protocol) and when ALC administration was started later on during treatment ("curative" protocol). Results: The ALC treatment significantly prevented the lowering of the mechanical nociceptive threshold when the administration started concomitantly and, respectively, with cisplatin, paclitaxel and vincristine as compared to each drug alone. Furthermore, when ALC administration was started later on during treatment, at well-established neuropathy, ALC was able to restore the mechanical nociceptive threshold within a few days. Finally, experiments indicated that ALC does not interfere with the antitumor effects of vincristine. Conclusion: Considering the absence of any satisfactory treatment currently available for CIPN in a clinical setting, these are important observations, opening up the possibility of using ALC to treat a wide range of patients who have undergone chemotherapy and developed sensory peripheral neuropathy.

Several effective antineoplastic drugs are severely neurotoxic and may induce the onset of disabling motor, sensory or sensorimotor neuropathy, depending on the type of agent administered. The pathogenesis of chemotherapy-induced peripheral neurotoxicity (CIPN) is still poorly understood. Cisplatin, the first platinum-derived drug to be used in clinical practice, induces a severe and dose-limiting sensory neuropathy due to damage of the primary sensory neurons of the dorsal root ganglia (DRG) (1) where it forms DNA adducts. Taxanes are another family of neurotoxic antineoplastic agents. The major mechanism of their antineoplastic action (i.e. enhancement of tubulin polymerization) is also likely to be one of the mechanisms at the basis of neurotoxicity, although, given the significant distribution of taxanes in the DRG, antitubulin action on the peripheral nerves is probably not the only mechanism of their neurotoxicity (2). Vinca alkaloids are also antitubulin agents, but with a different mechanism from taxanes, acting by dismantling the cellular cytoskeleton (3).

Acetyl-L-carnitine (ALC) is a member of the family of carnitines, a group of natural compounds which have an essential role in intermediary metabolism (4-7), but there is also experimental evidence that ALC has a neuroprotective action on both the central and peripheral nervous system (8-13).

In this study, we tested the hypothesis that ALC may have a protective and curative role on chemotherapy-induced hyperalgesia in vivo in animal models of cisplatin-, paclitaxel- and vincristine-induced neuropathy. In addition, the possible interaction between ALC and the antineoplastic action of vincristine was assessed.

Materials and Methods

Animal husbandry. The animals were housed in a limited access animal facility, at 22±2°C and 55±10% humidity, respectively. Artificial lighting provided a cycle of 12 hours light/12 hours dark (7 a.m.–7 p.m.). The care and husbandry of the animals were in accordance with European Directives no. 86/609, and with Italian D.L. 116, January 27th, 1992. All experiments were approved by the Sigma-Tau veterinarian.
Neurotoxicity experiments. CIPN was induced in different groups of rats following different experimental paradigms, as described in detail in Table I. Briefly, paclitaxel (1.2 mg/kg i.p. 6 times weekly for a total of 25 administrations), cisplatin (2 mg/kg i.p. twice weekly for a total of 5 administrations) or vincristine (0.150 mg/kg i.p. 3 times weekly until the end of the experiment) were administered to Wistar rats 3-4 months old (Charles River, Calco, Italy). Control animals were treated with vehicle alone. ALC was dissolved in sterile saline and in all the neuroprotection experiments was administered at a dose of 100 mg/kg/day s.c.

The effect of ALC was evaluated both when its administration was started together with the administration of antinecancer drugs ("preventive" protocol) and when ALC administration was started later on during treatment ("curative" protocol).

Behavioral experiment. The Randall-Selitto paw-withdrawal test was used to assess mechanical hyperalgesia (14). 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 (g) which induced rat paw withdrawal. The rats were habituated to the testing procedure during the week prior to the experiments (15). The test was conducted before treatment (baseline), during the administration of antineoplastic drugs and in the follow-up period, i.e. when toxic drugs were withdrawn while ALC treatment was still ongoing.

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

Results

Neurotoxicity studies

Paclitaxel model. The administration of paclitaxel induced a significant lowering of the mechanical nociceptive threshold compared to the control group (p=0.03).

Figure 1A shows the effects of repeated ALC co-treatment in this experimental model of paclitaxel-induced hyperalgesia in rats. The ALC treatment significantly prevented the lowering of the mechanical nociceptive threshold when its administration was started together with paclitaxel as compared to paclitaxel...
alone \((p=0.04)\). Figure 1B shows the effects of repeated ALC treatment when the treatment was started 15 days from the first paclitaxel treatment in the same experimental model of paclitaxel-induced hyperalgesia. ALC treatment was able to restore the mechanical nociceptive threshold within a few days \((p<0.05)\). The paw-withdrawal threshold values obtained in the paclitaxel-ALC group were not significantly different from those observed in the vehicle-treated animals.

Figure 1 (A, B). ALC and paclitaxel-induced neuropathy Randall-Selitto paw-withdrawal test. Mechanical nociceptive threshold score.
Cisplatin model. The administration of cisplatin induced a significant lowering of the mechanical nociceptive threshold compared to the control group ($p=0.001$). Figure 2A shows that ALC had a significant preventive effect, reducing the effect on the mechanical nociceptive threshold when its administration was started together with cisplatin as compared to cisplatin alone ($p=0.001$). Figure 2B shows the effects of repeated ALC treatment when the treatment was started 25 days from the first cisplatin treatment. Also in this experimental model, ALC treatment restored the mechanical nociceptive threshold within a few days of treatment ($p<0.001$).

Vincristine model. The administration of vincristine caused a significant lowering ($p<0.001$) of the mechanical nociceptive threshold compared to the control group. Figure 2B shows the effects of repeated ALC treatment when the treatment was started 25 days from the first vincristine treatment. Also in this experimental model, ALC treatment restored the mechanical nociceptive threshold within a few days of treatment ($p<0.001$).
nociceptive threshold compared to the control group. The simultaneous administration of ALC and vincristine prevented this reduction as opposed to when vincristine alone was administered ($p \leq 0.001$, Figure 3A). Figure 3B shows the effects of ALC treatment started 15 days from the first vincristine treatment; it demonstrates that ALC was rapidly able to restore the mechanical nociceptive threshold ($p < 0.001$) so that the paw-withdrawal threshold values obtained in the vincristine-ALC group were superimposable on those observed in the vehicle-treated animals.

Figure 3 (A, B). ALC and vincristine-induced neuropathy Randall-Selitto paw-withdrawal test. Mechanical nociceptive threshold score.
Oncology study

Antiproliferative effects of vincristine in combination with ALC. The antiproliferative activity of vincristine alone and in combination with a high concentration of ALC was evaluated in a panel of human tumor cell lines (Table II). In all tested cell systems, vincristine showed marked cytotoxic potency. The most sensitive cell lines were the human cell lines IGROV-1 and A2780 ovarian carcinomas, HeLa cervix uteri, NCI-H460 non-small cell lung carcinoma and the murine EL-4 T-lymphoma with IC50 in the range between 0.03 and 0.08 µg/ml, followed by LoVo and HT-29 colon carcinomas and PC3 prostate carcinoma (IC50 was 0.1 µg/ml).

ALC alone was not cytotoxic for the cells (IC50 >200 µg/ml). Moreover, the same concentration of ALC added to cells together with vincristine did not influence its growth-inhibitory effect on all human tumor cell lines tested. Only on the NCI-H460 and PC3 tumor cell lines did ALC slightly increase the cytotoxicity of vincristine, ranging from 1.6- to 2.6-fold (Table II).

Antitumor activity studies. On EL-4 lymphoma-bearing mice, vincristine showed significant antitumor activity at the therapeutic dose of 0.5 mg/kg i.p. (qdx5/wx2w), starting the treatment one day after the tumor injection, since it prolonged lifespan by 36% (p<0.001) (Table III).

Table II. Cytotoxicity of vincristine alone and in combination with ALC on different human tumor cell lines and a murine cell line.

<table>
<thead>
<tr>
<th>Tumor cell line</th>
<th>ALC</th>
<th>Vincristine</th>
<th>ALC+ Vincristine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC50 (µg/ml)±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HUMAN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovarian ca.</td>
<td></td>
<td></td>
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<tr>
<td>IGROV-1 &gt;200</td>
<td>0.03±0.003</td>
<td>0.03±0.003</td>
<td></td>
</tr>
<tr>
<td>A2780 &gt;200</td>
<td>0.04±0.004</td>
<td>0.04±0.004</td>
<td></td>
</tr>
<tr>
<td>Cervix uteri ca.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HeLa &gt;200</td>
<td>0.06±0.009</td>
<td>0.07±0.01</td>
<td></td>
</tr>
<tr>
<td>NSCLC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI-H460 &gt;200</td>
<td>0.085±0.005</td>
<td>0.032±0.003**</td>
<td></td>
</tr>
<tr>
<td>Colon ca.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT-29 &gt;200</td>
<td>0.1±0.01</td>
<td>0.078±0.01</td>
<td></td>
</tr>
<tr>
<td>LoVo &gt;200</td>
<td>0.1±0.04</td>
<td>0.1±0.02</td>
<td></td>
</tr>
<tr>
<td>Prostate ca.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC3 &gt;200</td>
<td>0.12±0.07</td>
<td>0.076±0.006**</td>
<td></td>
</tr>
<tr>
<td>MURINE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-lymphoma EL-4 &gt;200</td>
<td>0.026±0.003</td>
<td>0.029±0.004</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.01 vs vincristine (ALLFIT).

Table III. Effect of vincristine i.p. (qdx5/wx2w) in combination with ALC p.o. (qdx14) on median survival time in C57/BL6J male mice bearing a transplantable EL-4 T-lymphoma.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>BWL%a max</th>
<th>MSTb (range days)</th>
<th>ILS%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>/</td>
<td>14</td>
<td>(13-15)</td>
<td>/</td>
</tr>
<tr>
<td>ALC</td>
<td>100</td>
<td>0</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>Vincristine</td>
<td>0.5</td>
<td>5</td>
<td>19***</td>
<td>36</td>
</tr>
<tr>
<td>ALC+ Vincristine</td>
<td>+0.5</td>
<td>11</td>
<td>18*</td>
<td>28</td>
</tr>
</tbody>
</table>

*BWL% = maximum body weight loss during the experimental period. *MST = median survival time (days). *Controls, vehicle-treated group. **p<0.001 and *p<0.05 versus vehicle (Mann-Whitney)

ALC (100 mg/kg p.o.), administered daily for 14 days by itself, had no antitumor activity. The combination of ALC with vincristine did not influence the antitumor effects of vincristine (ILS=28% vs 36%) (Table III).

Discussion

The treatment of chemotherapy-induced neuropathy still represents an unsolved clinical problem. In fact, in most cases the treatment of the symptoms induced by chemotherapy is based on the empirical use of antiepileptic drugs or of antidepressants (17).

In our experiment, we used different animal models to investigate the possibility of administering ALC to protect from or to cure cisplatin-, paclitaxel- or vincristine-induced peripheral neuropathy. Moreover, on several tumor lines and in in vivo models, we demonstrated that ALC did not interfere with vincristine antitumor activity, a finding which is in agreement with the results already obtained in cisplatin and paclitaxel in vitro and in vivo tumor models (18).

To assess the neuroprotective action of ALC, we used the Randall-Selitto paw-withdrawal test, since this method is considered a reliable instrument for assessing allodinia in experimental models (14, 15) and, moreover, sensory changes are prominent during the clinical use of all the antineoplastic drugs evaluated in our study.

The use of behavioral tests in neurotoxicology raises several issues, such as the translation of their results to humans, the comparison with neurophysiological and pathological results and the selection of the most appropriate method to investigate a specific problem. The paw-withdrawal test is appropriate in our experimental paradigm since it explores one of the symptoms most frequently reported by treated...
patients. Moreover, the results obtained with this behavioral test in the cisplatin and paclitaxel models when ALC is co-administered are in agreement with the neurophysiological and pathological results already reported in the literature (18), thus giving a direct confirmation of its reliability.

The sum of the results obtained in our cisplatin and paclitaxel neurotoxicity experiments confirmed that ALC co-administration reduced their toxicity (18), and also demonstrated that this protective effect was similarly evident in the vincristine model.

Moreover, ALC was also very effective in "curing" chemotherapy-induced peripheral neuropathy once it was administered to rats already showing evidence of peripheral neuropathy.

ALC is very active on intermediary metabolism (4-7) and the neuroprotective activity of this molecule was demonstrated on both the central and peripheral nervous systems. In fact, the administration of ALC reduces the severity of experimental diabetic neuropathy (8-10), enhances motorneuron survival after axotomy (11), modulates the control of the nerve growth factor level in the CNS of adult rats following total fimbria-fornix transection (12) and the rate of transcription of the gene coding for the p75NGFR in the basal forebrain and cerebellum of aged rats (13).

In the specific field of neuroprotection from chemotherapy-induced peripheral neurotoxicity, ALC has already been tested in several "protective" models (18) and it is remarkable that, in all these studies, no reduction in antitumor activity was ever demonstrated. However, the results of the present study represent the first demonstration in animal models that ALC is also active in a "curative" way, i.e. once peripheral neurotoxicity has already been established.

In view of the absence of any satisfactory treatment currently available for chemotherapy-induced symptoms in a clinical setting, this observation is important since it opens up the possibility of using ALC to treat a wide range of patients who have undergone chemotherapy and developed sensory peripheral neuropathy.

Acknowledgements

We thank Patrizia Tobia, Angelo Marconi, Silvio Zavatto, Maria Grazia Scrocco and Mauro Comuzio for their excellent technical assistance.

References


Received December 28, 2004
Accepted February 17, 2005