Mitoxantrone dihydrochloride, a synthetic anthraquinone, is a potent antineoplastic agent and active substance of REFADOR Inj. PLIVA-LACHEMA. The chemical structure and chemical name are:

\[
\text{OH} \quad \text{H} \quad \text{N} \quad \text{NH} \quad \text{OH}
\]

\[
\text{OH} \quad \text{O} \quad \text{H} \quad \text{N} \quad \text{NH} \quad \text{2HCl}
\]

1,4-dihydroxy-5,8-bis\{2-[(2-hydroxyethyl)amino]ethylamino\} anthracene-9,10-dione dihydrochloride.

This active component of the preparation is manufactured by the Research Institute of Organic Synthesis (VÚOS) (17) Pardubice, Czech Republic.

Mitoxantrone (MX) can be used alone and in combination with other agents against various types of neoplasias, including solid tumours (8) and haematological malignancies (14,20). Toxic effects of antineoplastic therapeutics and acquired cell resistance to these agents, occurring in the course of therapy, are the limiting factors of successful cancer treatment (14,20).

Among substances used to give metabolic support, we tried preclinically to determine whether some L-carnitine derivatives, in combination with MX, could ameliorate host’s metabolic response to tumour processes. The aim was to document new possibilities of using a combination of chemotherapeutics with substances that modulate their therapeutic and toxicologic profiles and that could be of clinical importance as new antimour drugs and new therapeutic protocols.

In this work we investigated the therapeutic benefit of acetyl-L-carnitine (ALC) in combination with MX on a murine leukemia L1210 resistant to MX.

Materials and methods

Mitoxantrone (MX) (batch No 12/309 VÚOS) was purchased from the Research Institute of Pharmacy and Biochemistry.

Summary: Supportive care in tumour chemotherapy is a subject of intensive research. The complications of cytostatic therapy are a cause of extensive research of their pharmacological interactions and side effects. The immunologic and biochemical changes accompanying tumours are the factor that is most responsible for the worsening of the physiology of the host. Regimens containing carnitine and its acetyl/derivative are used in many cases, among others even for preventing hepatotoxicity. Our hypothesis was to verify the supporting metabolic effects of acetyl-L-carnitine hydrochloride (ALC) in combined therapy with mitoxantrone (MX) and hepatotoxic cytostatic drugs including alkylating agents. This present report describes the effect of ALC in combination with MX on B6A/J male mice bearing a transplantable L1210 leukemia resistant to MX. The criterion for evaluation of effect was the length of survival time of experimental animals. The proportional-hazards model quadratic in the drug dose (7) was used for survival time evaluation and optimal dose calculation. The hazard functions and the index of relative hazard were determined using Weibull distribution after logarithmic transformation of the entered data in each particular group. The dose-response curve was represented by a second-degree polynomial without absolute term. The combination therapy revealed that the optimal dose of ALC was 186 mg/kg s.c. This relation is shown in Fig. 1. A significant effect of ALC (s.c.) in combined therapy with MX (6 mg/kg i.v.) given to animals bearing an experimental form of leukemia L1210/MX resistant to MX was proven at a level of probability ps 0.001.

The effect of ALC in monotherapy was not demonstrable.

Key words: Mitoxantrone dihydrochloride (MX); Acetyl-L-carnitine hydrochloride (ALC); Protective effect and L1210 leukemia

ORIGINAL ARTICLE

EFFECT OF ACETYL-L-CARNITINE ON LEUKEMIA L1210 RESISTANT TO MITOXANTRONE

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Submitted May 2000.
Accepted July 2000.

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ACTA MEDICA (Hradec Králové) 2000;43(4):125-128
in Prague. It is a dark blue hygroscopic crystalline substan-
cce, soluble in water, physiological saline and isonicotin des-
trose solution, with a molecular weight of 517.41.

Acetyl-L-carnitine hydrochloride, a white hygroscopic
 crystalline substance, with a molecular weight of 239.70 with
 CAS registry No 5080-50-2. It was a kind gift from
 Lonza LTD Organic Chemicals Basle, Switzerland.

The anitneoplastic activity of MX combined with ALC,
 was evaluated in vivo on a transplanteable L1210 leukemia
 variant selected for resistance to MX (L1210/MX).

Methods

DBA/2 male mice from Velaz a.s. weighing 21.3-24.8 g
 were used. L1210 cell suspension was intraperitoneally ino-
culated (2.10^7 cells from the ascitis fluid in 0.2 ml of phy-
siological saline per mouse). 80 DBA/2 mice bearing this
 tumour transplant were divided into 8 groups, a control
 group and 7 test groups of 10 animals. Animals in the test
 groups were treated with intravenous (i.v.) or intraperitone-
 al (i.p.) administrations of MX in a single dose of 6 mg/kg
 combined with different doses of ALC ranging from 50 -
 200 mg/kg. ALC was administered either subcutaneously
 (s.c.) or intraperitoneally (i.p.).

The proportional-hazards model quadratic (a) in the
drug dose (7) was used for evaluation of the survival time
and optimal dose calculation.

\[
\lambda(t) = \lambda_0(t) \exp(\beta_1 x + \beta_1^2 x^2)
\]

where \(\lambda_0\) and \(\lambda_1\) are the hazard functions at time \(t\),
A is the dose of mg/kg in combination with a single dose
of MX, \(\beta_1\) and \(\beta_1^2\) are the coefficients of the second-degree poly-

In vivo effect of combination therapies of MX 6 mg/kg
(i.v.) and ALC at doses of 200, 100 and 50 mg/kg
(s.c.) or (i.p.) on DBA/2 mice bearing a transplanteable leu-
kenoma L1210 variant resistant to mitoxantrone. Treatment
1x1 began the first day following the intraperitoneal ino-
culation of the tumour on day 0. The table shows the survival
days and compares the effect of MX administered (i.v.) ver-
sus (i.p.).

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Dose</th>
<th>Survival days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>——</td>
<td>5 6 15 18 19 19 19 19 19</td>
</tr>
<tr>
<td>MX</td>
<td>10</td>
<td>5 i.v.</td>
<td>6 9 9 12 12 15 17 19 20 20</td>
</tr>
<tr>
<td>ALC</td>
<td>10</td>
<td>0</td>
<td>10 13 14 15 16 17 18 18 20 20</td>
</tr>
<tr>
<td>MX</td>
<td>10</td>
<td>0</td>
<td>10 13 14 15 16 17 18 18 20 20</td>
</tr>
<tr>
<td>ALC</td>
<td>10</td>
<td>200 s.c.</td>
<td>14 16 17 17 17 18 18 18 18 18</td>
</tr>
<tr>
<td>MX</td>
<td>10</td>
<td>4 i.v.</td>
<td>5 9 11 13 16 17 18 18 18 18</td>
</tr>
<tr>
<td>ALC</td>
<td>10</td>
<td>100 s.c.</td>
<td>9 11 12 16 17 18 18 18 18 18</td>
</tr>
<tr>
<td>MX</td>
<td>10</td>
<td>6 i.v.</td>
<td>16 26 33 33 33 35 35 39 39 39</td>
</tr>
<tr>
<td>ALC</td>
<td>10</td>
<td>100 s.c.</td>
<td>6 26 33 33 33 35 35 39 39 39</td>
</tr>
</tbody>
</table>

The proportional-hazards model quadratic (b) in the
drug dose (7) was used for evaluation of the survival time
and optimal dose calculation.

\[
\lambda(t) = \lambda_0(t) \exp(\beta_1 x + \beta_1^2 x^2)
\]

where \(\lambda_0\) and \(\lambda_1\) are the hazard functions at time \(t\),
A is the dose of mg/kg in combination with a single dose
of MX, \(\beta_1\) and \(\beta_1^2\) are the coefficients of the second-degree poly-

Dose-response curve for acetyl-L-carnitine hydrochloride (ALC).
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The antineoplastic activity of MX combined with ALC,

Methods

DBA/2 male mice from Velaz a.s. weighing 21.3–24.8 g

were used. L1210 cell suspension was intraperitoneally ino-

culated (2.10^6 cells from the ascites fluid in 0.2 ml of phy-

Table: Antitumour activity of mitoxantrone dihydrochlo-
idine (i.v. or i.p.) and acetyl-L-carnitine hydrochloride (s.c. or

Fig. 1: Dose-response curve for acetyl-L-carnitine hydro-

chloride (ALC). The figure shows the dependence of the index of relative haza-

The length of survival of experimental animals bearing a

parametric test, p≤0.05).

The experiment demonstrated a statistically significant

Discussion

For most chemotherapeutic regimens, there is a rela-

tionship between the dose of drug given and the likelihood of

cancer cell kill. However, as drug doses are increased, toxicity in-

creases. As opposed to many other classes of drugs, the dose

of an antineoplastic agent adequate to achieve tumour cell

kill often causes toxicity to normal tissues (15).

The primary toxicities of MX are similar to those seen

with the anthracyclines: myelosuppression, nausea, vomit-
ing and cardiac toxicity. Cancer cells are very effective in over-

coming the problem of resistance development, more than

one drug is generally used to treat a cancer (combination

chemotherapy). For instance, most of the topoisome-

rase II targeted drugs develop cellular resistance through

either a mutation in topoisomerase II, decreased topoisome-

rase II production or through production of p-glycopro-

tein conferring multidrug resistance (3,10). Drugs used in

combination therapies generally have different cellular tar-

gets to avoid development of drug resistant tumour cell li-

trants). First, the planar moiety can insert between base pairs

of DNA (intercalation). Application of drugs with this mecha-

nism to DNA strands leads to single-strand breaks and

double-strand breaks.

The anthracyclines appear to be the primary mecha-

nism by which this class of drugs causes damage to heart

tissue

The degree of severity of toxicity is a real challenge to

successful treatment. Toxicity modulating agents are now

clinically used.

Antineoplastic activity of MX combined with ALC was

evaluated on transplantable mouse L1210 leukemia in vivo.
The combination therapy has been investigated on a murine

model DBA/2.L1210 leukemia selected for resistance to

mitoxantrone. MX was administered at a single dose of

6 mg/kg i.v. or i.p. ALC was given in doses ranging from

50–200 mg/kg (Tab.1). Survival of the experimental ani-

mals was observed for 90 days following leukemia inocula-

tion.

For all tested groups, the statistical evaluation of the

functional dependence of survival on doses of MX i.v. alon-
e or in combination with ALC s.c. revealed that the F-

value (Fisher-Snedecor F-test used for evaluation of the

statistical significance of the model) 9.765 was greater than

the critical value for the combination of these two drugs at

0.001 level of probability (Tab.2). Thus ALC (s.c.) in combi-
nated therapy improved the therapeutic effect of MX dose
dependently and significantly. The dose-response function of

antenitumour activity of ALC in combination with MX 6 mg/kg i.v.
ix appears in Fig.1. The optimal dose of ALC in combina-
tion with MX was 186 mg/kg s.c.

Results

Antineoplastic activity of MX combined with ALC was

evaluated on transplantable mouse L1210 leukemia in vivo.

For all tested groups, the statistical evaluation of the

functional dependence of survival on doses of MX i.v. allo-

ne or in combination with ALC s.c. revealed that the F-

value (Fisher-Snedecor F-test used for evaluation of the

status of the statistical model)

-0.21414 0.04492 4.707 0.004 0.5985 9.765

Status: ALC active (p ≤ 0.001). Optimal dose 186 mg/kg s.c.

\beta = \text{estimated value of the parameter}

N = \text{number of evaluated subjects}

SD = \text{standard deviation}

r = \text{correlation coefficient}

\text{Fisher-Snedecor test value}

F = r^2 (N-1) / (N-p) p, number of parameters.

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Dose-response curve for acetyl-L-carnitine hydrochloride (ALC).

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Limited data are available concerning the mechanisms of tissue wasting and weight loss in cancer patients. Malnutrition associated with malignancy has been documented in many hospitalised cancer patients. This character-
istic state of malnutrition and progressive tissue wasting is referred to as cancer cachexia and is believed to result from a combination of decreased nutrient intake, altered energy expenditure and abnormal substrate utilisation. However, recent literature suggests that this response is also a result of complex metabolic alterations and not only a re-
sult of starvation (16,26). Thus the presence of cancer ap-
ppears to cause metabolic alterations in the host (22,26).
All these data suggest the probable beneficial role of
nourishment (i.e supportive) care in cancer treatment. When using ALM in combination with MX, we observed a sub-
stantial increase in length of survival of treated animals
compared to MX alone. Intravenous application seems to
be the best way of MX application. However intraperitone-
al application was demonstrated as effective in this experi-
ment. The contact with tumour cells in situ is probably necessary for the expression of the cytotoxic effect of
the drug.
Our hypothesis was not only to modulate the adverse ef-
fact of MX therapy but also to make a change in energetic balance in favour of the host. The clinical use of ALM as an adjuvant to MX and other antineoplastic agents may be
a useful contribution in improving the metabolic state.

Acknowledgements

Anna Kargerova and Jitka Sediva from the Research Institute for Pharmacy and Biochemistry Prague are ack-
nowledged for technical assistance in experiments involving
mice.

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