Introduction

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:

\[
\text{OH} \quad \text{O} \quad \text{HN} \quad \text{NH} \quad \text{OH}
\]

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). The toxic effects of anticancer 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, researchers are trying 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 that could be effective in the treatment of both solid and haematological malignancies, including solid tumours (8) and haematological malignancies (6,21). Toxic effects of anticancer therapeutics and acquired cell resistance to these agents, occurring in the course of therapy, are the limiting factors of successful cancer treatment (14,20).

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 (batch No 12/309 VÚOS) was purchased from the Research Institute of Pharmacy and Biochemistry.
combination therapies generally have different cellular targets to avoid development of drug resistant tumor cell lines. With commonly used dosages, approximately 80% and MX have been described. First, the planar moiety can insert between base pairs of DNA (intercalation). This results in DNA conformational changes, with subsequent inhibition of DNA replication (1). The antitumour activity of AC-LC is primarily due to the anthracyclines appears to be the primary mechanism by which this class of drugs causes damage to heart tissue. The critical value for the combination of these two drugs at 0.001 level of probability (Tab.2). Thus ALC (s.c.) in combination therapy improved the therapeutic effect of MX dose dependently and significantly. The dose-response function of murine ALC in combination with MX 6 mg/kg i.v. appears in Fig.1. The optimal dose of ALC in combination with MX was 186 mg/kg s.c.

The antitumour activity of mitoxantrone dihydrochloride (i.v. or i.p.) and AC-LC and antitumour activity (s.c. or i.p.) on L1210/MX leukemia bearing DBA/2 mice. The in vivo effect of combination therapies of MX 6 mg/kg (i.v.) or (i.p.) and ALC at doses of 200, 100 and 50 mg/kg (s.c. or i.p.) on DBA/2 mice bearing a transplantable leukemia L1210 variant resistant to mitoxantrone. Treatment 1x1 began the first day following the intraperitoneal inoculation of the tumour on day 0. The table shows the survival days and compares the effect of MX administered (i.v) versus (i.p.).

The degree of severity of toxic effect is a real challenge to successful treatment. Toxicity modulating agents are now clinically used.

Antitumour activity of mitoxantrone dihydrochloride (Tab. 1): Myelotoxicity is clinically managed by use of growth factors (i.v. or i.p.) and acetyl-L-carnitine hydrochloride (s.c. or i.p.) on L1210/MX leukemia bearing DBA/2 mice. Carnitine derivatives play a key role in regulating the flow of energetic substrates through cell membranes (9,11,23). A decrease of fatty acid oxidation due to impairment of beta-oxidation results in accumulation of triglycerides, therefore resulting into both microscopic fatty change and impaired hepatic ketogenesis (13).

The involvement of carnitine and its acetyl-derivative has been extensively reported. Biochemically, ALC acts as a carrier of fatty acids across the inner mitochondrial membrane (24). ALC enhances the utilisation of alternative sources of energy (1).

1.6 1.2 0.8 0.4 0.0 -0.4 -0.8 -1.2 -1.6 -2.4 -2.8

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

Fig. 1: Dose-response curve for acetyl-L-carnitine hydrochloride (ALC) in the study of survival of DBA/2 mice bearing leukemia L1210/MX resistant to mitoxantrone. The figure shows the dependence of the index of relative hazard R on doses of ALC. The optimal dose of ALC in combination with MX (6mg/kg i.v.) was found to be 186 mg/kg s.c.

The length of survival of experimental animals bearing a variant form of leukemia resistant to MX was not statistically different at the dose of MX 6 mg/kg i.v. compared to control group (non-parametric test, p 0.05). In combined therapy with MX (i.p.), ALC at dose of 100 mg/kg (i.p.) did not improve the therapeutic effect of MX (non-parametric test, p0.05).

The experiment demonstrated a statistically significant effect of ALC (s.c.) when combined with MX i.v., whereas the effect of ALC (s.c.) alone was not proven.

Discussion

For most chemotherapeutic regimens, there is a relationship between the dose of drug given and the likelihood of obtaining a therapeutic outcome (i.e. higher doses give greater tumour cell kill). However, as drug doses are increased, toxicity increases. 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, vomiting and cardiac toxicity. Cancer cells are very effective in developing biochemical mechanisms that allow for cellular resistance to a particular antineoplastic agent. In order to overcome the problem of resistance development, more than one drug is generally used to treat a cancer (combination chemotherapy). For instance, most of the topoisomerase II targeted drugs develop cellular resistance through either a mutation in topoisomerase II, decreased topoisomerase II production or through production of p-glycoprotein conferring multidrug resistance (3,10). Drugs used in combination therapies generally have different cellular targets to avoid development of drug resistant tumour cell lines.

Four potentially significant effects of anthracyclines and MX have been described. First, the planar moiety can insert between base pairs of DNA (intercalation). This intercalation was originally felt to be the mechanism for anthracycline cytotoxicity. Second, these agents inhibit topoisomerase II (23). This appears to be the primary mechanism for tumour cytotoxicity. Third, the anthracyclines modify the ability of nuclear helicases to dissociate duplex DNA into single DNA strands, thus hindering the process of strand separation (2). Finally, all clinically active anthracyclines are antihormones. As true of all quinones, the anthracyclines can undergo one and two electron reduction producing reactive compounds which damage macromolecules and lipid membranes (19). It is now known that reduced metals (such as iron) are critical components in the formation of these reactive intermediates (5).

Mycoplasma is clinically managed by use of growth factors or by alogenic bone marrow transplantation (ABMT), whereas cardiotoxicity, hepatotoxicity could be circumvented by use of carnitine and its acetyl derivative known to have both cardioprotective and hepatoprotective effects.

Our hypothesis was to verify the beneficial role of ALC in modulating the adverse effects of mitoxantrone activity.

Over the past decades, numerous reports have shown the beneficial effect of carnitine and its acetyldervative in animals and humans. The involvement of carnitine and its acetyldervative has been extensively reported. Biochemically, ALC acts as a carrier of fatty acids across the inner mitochondrial membrane (24). ALC enhances the utilisation of alternative sources of energy (1). ALC has been shown to have both cardioprotective and hepatoprotective effects.

Our hypothesis was to verify the beneficial role of ALC in modulating the adverse effects of mitoxantrone activity. The 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 animals was observed for 90 days following leukemia inoculation. For all tested groups, the statistical evaluation of the functional dependence of survival on doses of MX i.v. alone 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 combination therapy improved the therapeutic effect of MX dose dependently and significantly. The dose-response function of murine ALC in combination with MX 6 mg/kg i.v. appears in Fig.1. The optimal dose of ALC in combination with MX was 186 mg/kg s.c.
in Prague. It is a dark blue hygroscopic crystalline substan-cible in water, physiologically saline and isotonic de-score 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 antineoplastic activity of MX combined with ALC, was evaluated in vivo on a transplantable 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 inocu-lated (2×10³ cells from the ascites fluid in 0.2 ml of ph-y-ysiological 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 intraperitoneal (i.p.) administrations of MX in a single dose of 6 mg/kg combined with different doses of ALC ranging from 30 - 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.

\[ \lambda(t) = \lambda_0(t) \exp(b_1 + b_2t^2) \]  

where \( \lambda_0 \) and \( \lambda \) are the hazard functions at time \( t \), \( b_1 \) and \( b_2 \) are coefficients of the second-degree poly-nomial (a) without absolute term. Hazard functions and the index of relative hazard (b) were calculated using parameters of Weibull distribution after logarithmic transformation of entered data of each partic-ular group. The dose-response curve was represented by a second-degree polynomial without absolute term.

Results

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. allo-ne or in combination with ALC s.c. revealed that the F-va-lue (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 probabiliy (Tab.2). Thus ALC (s.c.) in com-bined therapy improved the therapeutic effect of MX dose dependently and significantly. The dose-response function of ALC in the combination of MX with 6 mg/kg i.v. appears in Fig.1. The optimal dose of ALC in combina-tion with MX was 186 mg kg s.c.

Tab. 1: Antitumour activity of mitoxantrone dihydrochlori-de (i.v. or i.p.) and acetyl-L-carnitine hydrochloride (s.c. or i.p.) on L1210/MX leukemia bearing DBA/2 mice.

| Group     | Dose       | Survival days |
|-----------|------------|---------------|
| Control   | 10         | ——–          |
| MX 10     | 6 i.v.     | 4 6 9 10 12 13 17 18 20 29 |
| ALC 100   | s.c.       | 14 16 17 18 19 20 21 22 23 24 |
| MX 10     | 6 i.v. 200 s.c. | 7 9 11 12 13 14 15 16 17 18 |
| MX 10     | 6 i.v. 100 s.c. | 5 7 9 10 11 12 13 14 15 16 |
| MX 10     | 6 i.p. 50 s.c. | 8 10 12 13 14 15 16 17 18 19 |
| MX 10     | 6 i.p. 100 s.c. | 3 5 7 8 9 10 11 12 13 14 |
| MX 10     | 6 i.p. 150 s.c. | 1 3 5 7 9 10 12 14 16 18 |
| MX 10     | 6 i.p. 200 s.c. | 0 2 4 6 8 10 12 14 16 18 |
| MX 10     | 6 i.p. 250 s.c. | 0 2 4 6 8 10 12 14 16 18 |
| MX 10     | 6 i.p. 300 s.c. | 0 2 4 6 8 10 12 14 16 18 |
| MX 10     | 6 i.p. 350 s.c. | 0 2 4 6 8 10 12 14 16 18 |

For most chemotherapeutic regimens, there is a rela-tionship between the dose of drug given and the likelihood of obtaining a therapeutic effect (i.e. higher doses give greater tumour cell kill). However, as drug doses are increased, toxicity in-creases. As opposed to many other classes of drugs, the dose of the 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 developing biochemical mechanisms that allow for cellular resistance to a particular antineoplastic agent. In order to overcome the problem of resistance development, more than one drug is generally used to treat a cancer (combina-tion chemotherapy). For instance, most of the topoiso-merase II targeted drugs develop cellular resistance through either a mutation in topoisomerase II, decreased topoisomerase 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 lines.

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Carnitine derivatives play a key role in regulating the flow of energetic substrates through cell membranes (12). A decrease of fatty acid oxidation due to impair-ment of carnitine dependent mechanisms results in an im-paired hepatic ketogenesis. When the carnitine dependent mechanisms are impaired, fatty acid oxidation is reduced and triglycerides accumulate, therefore resulting into both microscopic fatty change and impaired hepatic ketogenesis (13).
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 characteristic 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 result of starvation (16,26). Thus the presence of cancer appears to cause metabolic alterations in the host (22,26).

All these data suggest the probable beneficial role of nutritive (i.e. supportive) care in cancer treatment. When using ALA in combination with MX, we observed a substantial increase in length of survival of treated animals compared to MX alone. Intravenous application seems to be the best way of MX application. However intraperitoneal application was demonstrated as effective in this experiment. 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 effect of MX therapy but also to make a change in energetic balance in favour of the host. The clinical use of ALA as an adjuvant to MX and other antineoplastic agents may be a useful contribution in improving the metabolic state.

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ORIGINAL ARTICLE

LONG TERM EFFECT OF FIBRE SUPPLEMENT AND REDUCED ENERGY INTAKE ON BODY WEIGHT AND BLOOD LIPIDS IN OVERWEIGHT SUBJECTS

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Summary: A weight-reducing potential has been ascribed to high dietary fibre intake. To investigate the practical reliability of this hypothesis, fifty-three moderately overweight females (BMI > 27.5 kg/m²) on reduced energy intake (1200kcal/day) were treated for 24 weeks with a fibre supplement on a randomly, double-blind, placebo-controlled basis. The fibre was administered as an initial dose of 6 g and a maintenance dose of 4 g. Body weight and blood pressure were recorded weekly during the first 3 months and thereafter every second week. Blood samples were drawn at start and at end of the study. Initial body weights were 75.6 ± 1.6 kg in the fibre group versus 75.5 ± 1.6 kg in the placebo group. After treatment, mean weight loss in the fibre group was 8.0 kg versus 5.8 kg in the placebo group (p < 0.05). Systolic and diastolic blood pressures were significantly reduced in both groups without differences between the groups. Serum concentrations of cholesterol, triglycerides and uric acid were significantly reduced in the group with reduced energy intake, whereas no additional effect was observed when fibre was included. Serum concentrations of potassium and sodium did not change significantly. The results suggest that a dietary fibre supplement in combination with a hypocaloric diet is of value as an adjunct in the management of overweight.

Key words: Dietary fibre, Obesity, Weight reduction, Blood pressure

Introduction

Material and methods

Subjects

Sixty healthy, mildly overweight females who volunteered to participate in the long term study were recruited by announcement in the local newspaper. The inclusion criteria were age 18-67 year and body mass index (BMI) > 27.5 kg/m². Patients with serious cardiac, renal and hepatic diseases were excluded. Patients with a history of gastrointestinal disease, type 1 diabetes, pregnancy, and patients treated with antacids, diuretics, β-blockers, bulk laxatives, anorectics, and oral contraceptives were also excluded.

Study design

All subjects were randomised to two groups according to BMI, the fibre group and the placebo group. Fibre tablets (Farmaa Food, Copenhagen, Denmark) and placebo tablets (identical in taste and appearance as fibre during prescribed three times daily, 6 tablets to be taken 15 min before meals with 250 ml of water and 4 tablets to be taken at 3 p.m. for 8 weeks (high dosage). The dosage was then reduced to 5 tablets prescribed three times daily for the rest of the treatment period (maintenance dosage). The tablets contained a mixture of fibres from grain and citrus and consisted of...