Article is available online at http://www.webio.hu/por/2003/9/2/0096

ARTICLE

Trace Elements Improve Survival of DTIC-Treated Mice with Overt Liver Metastases of Lewis Lung Carcinoma

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Trace elements have been previously shown to have specific antimetastatic effects in a mouse 3LL-HH liver metastasis model. Here we have analyzed the effect on the survival of animals with liver metastases. Trace elements administered per os at 500-5000 μg/kg/day did not affect the survival of animals with liver metastases. However, when trace element treatment was combined with dacarbazine (DTIC) administration, the survival of animals was significantly improved (55%). This effect was specific for DTIC since trace elements did not influence the effect of 5-fluorouracil on survival in this liver metastasis model. These data and those found in the literature all suggest that trace elements can specifically modulate the antitumoral/antimetastatic effects of chemotherapeutic agents. (Pathology Oncology Research Vol 9, No 2, 96–99, 2003)

Keywords: 3LL-HH tumor, mice, trace element mixture, liver metastasis, survival, 5-FU, DTIC

Introduction

Tumor progression is a complex process characterized by unique interactions between tumor cells and the host extracellular matrix and host cells. The result of these interactions is the colonization and growth of tumor cells in the form of metastases in various organs ultimately leading to the death of the host. Though successful treatment of the tumors at the primary site can be achieved by surgery and/or radio- and chemotherapy, intervention against the disseminated tumor is much less efficient. These interventions have to be able to target not only the tumor cells but their intercellular communications occurring between the tumor cells and the host tissues.

Trace elements are essential for normal cellular processes, including gene transcription, enzyme activities, and cell adhesions, but their role in cancer is still largely unknown. Earlier studies discovered that the immunomodulatory activity of trace elements was beneficial for the host carrying experimental tumors[1,2] and such treatment inhibited tumor growth as well.[3] We have demonstrated that trace element administration not only inhibited tumor growth but specifically inhibited liver metastatization of 3LL-HH tumor cells.[4] Detailed analysis identified Zn as one of the active components among trace elements responsible for the antimetastatic effect. However, none of these previous studies tested the effect of trace elements on the survival of animals bearing disseminated tumors, although clinically this effect would be the most important. Accordingly, in this study we have performed survival experiments using a highly aggressive liver metastatic animal model, 3LL-HH, to test trace element administration. Furthermore, we also tested the effect of trace elements on 5-FU and DTIC, two common constituents of combined chemotherapeutic modalities.

Materials and Methods

We have used a highly metastatic murine lung carcinoma line, 3LL-HH, selected for liver metastatic potential.[5] The progression in this ***in vivo*** model is largely independent from the immune system of the host, since this tumor is immunoresistant.[6] Trace element preparations are available commercially and they are part of our everyday diet, therefore we have chosen a trace element preparation frequently used as food additive (Trace element preparation, Béres

Received: April 10, 2003; *accepted:* May 25, 2003

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Ltd., Budapest, Hungary).[2] It contains the most important trace elements Fe, Zn, Mg, Mn, Cu in form of SO_4 complex as well as several rare elements (Ni, F, Co, Va).[4] Trace elements were administered into the stomach of the animals daily at doses of 500 and 5000 μg/kg body weight. In certain experiments trace element administration was combined with chemotherapy. 5-fluorouracil (5-FU, ICN, Hungary) was used in a single dose administered on the 7th postinoculation day (50 mg/kg i.p.). Dacarbazine (DTIC, Pliva, Zagreb, Croatia) was administered for 2 weeks (starting on the 4th postinoculation day) at a 60 mg/kg dose.

As an experimental model, 3LL-HH tumor cells were injected into the spleen of animals when the primary tumor was developed and parallel to this regular liver metastases have been produced within 2 weeks.[5] To mimic the human situation, three days following tumor cell inoculation the spleen was removed and liver metastases develop in the absence of the primary tumor (surgical model). At termination of the experiments animals were anesthesized by Nembutal overdose and the liver was analyzed macroscopically and microscopically. 3LL-HH tumor is a highly aggressive tumor, where 10^2 tumor cells can kill the host within the experimental period,[5] therefore a low tumor burden was produced throughout the experiments by injecting $5x10^2$ cells into the spleen, when the number of liver metastases were in the range of 20-50. In the majority of the experiments survival of animals carrying 3LL-HH tumor metastases was determined.

Statistical analysis was performed by ANOVA or Mantel-Cox general savage methods.

Results

Effect of trace elements on the survival of animals with metastatic tumor

In the surgical metastasis model, the primary tumor site was removed at an early phase of tumor progression (on the 3rd postinoculation day) following intrasplenic injection of 3LL-HH cells. Trace element administration to animals was started after the removal of the spleen primary and was carried out for 2 weeks. As was observed before,[4] the per os trace element treatment inhibited the formation of liver metastases of 3LL-HH cells and the 5000 μg/kg/day dose produced significant result ($p<0.05$) (***Figure 1a***). However, when we repeated the experiment, nsing survival as an endpoint of the effect (we have determined the survival of the animals), we found no significant effects of trace elements (***Figure 1b***).

Effect of trace elements on the efficacy of chemotherapy

Previous studies indicated a significant but relatively modest antitumor effect of trace elements in experimental animals. In the following experiments we tried to model

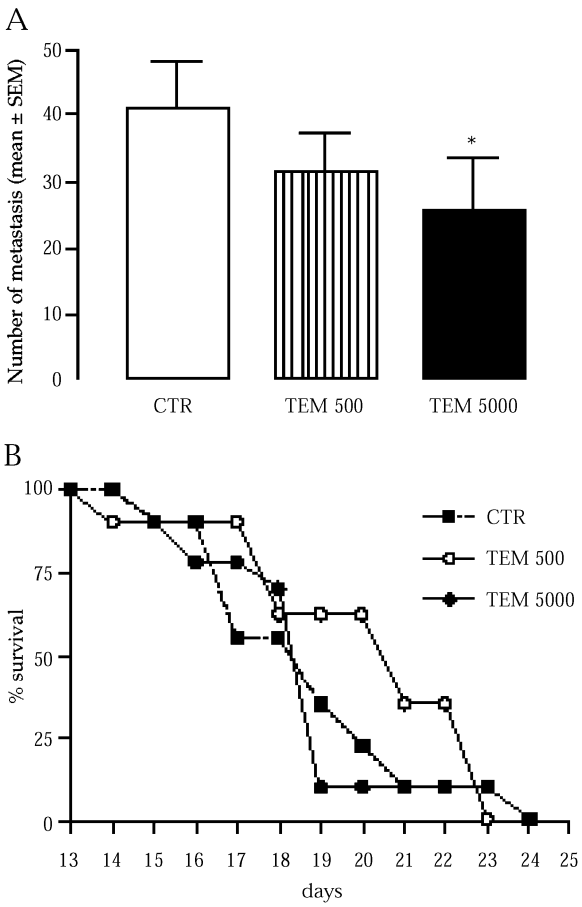

Figure 1. *Effect of trace element administration on mice with liver metastases of 3LL-HH tumor (surgical model).* ***(A)*** *Effect on the development of liver metastases. Animals were treated with trace elements (TEM) for 14 days following the removal of the primary tumor at 500 and 5000 μg/kg doses and the experiment was terminated. *p<0.05, ANOVA.* ***(B)*** *Effect of TEM administration on the survival of mice with liver metastases. Animals were treated as in case of A. Data are expressed in % of surviving animals (each group contained a minimum of 10 animals).*

the clinical situation where regular chemotherapy is supplemented by daily administration of trace elements using the same surgical spleen-liver model as above. We have used two commonly used agents, 5-FU and DTIC, exerting different antimetastatic effects in 3LL-HH cells;[7] which are less sensitive to 5-FU than to DTIC. Instead of artificially interrupting the biological process at the end of the 2nd week, we have used the survival of the animals as surrogate marker of the bioactivity of the combination therapy.

5-FU is a common component of several clinically used chemotherapeutic regimes and is extensively used to treat breast and colon cancers. Animals carrying 3LL-HH tumor in the spleen were treated with a single dose of 5-FU (50

mg/kg, i.p.) on the 7th postinoculation day. Trace elements were administered within the first 2 weeks with a high dose of 5000 μg/kg/day. Single doses of 5-FU treatment or trace elements did not cause significant increase in survival of animals and the combination of the two regimes did not change this trend either (*Figure 2a*).

DTIC is the common chemotherapeutic agent in the case of human melanoma and 3LL-HH cells are sensitive,[7] therefore we next studied its effect in combination with trace elements. Similarly to the clinical situation, DTIC was administered i.p. daily at a dose of 60 mg/kg for 14 days. Trace elements were administered orally for the first 14 days of the experiment at 5000 μg/kg/day. DTIC treatment resulted in a 22% increase in the survival of the 3LL-HH tumor-bearing animals being at the border of statistical significance (p=0.067, *Figure 2b*). However, combination of the DTIC treatment with the trace element administration produced a significant (55%, p<0.05) increase in the survival of animals compared to the single DTIC treatment modality, suggesting that trace elements may promote the antitumor activity of DTIC in this tumor model (*Figure 2b*).

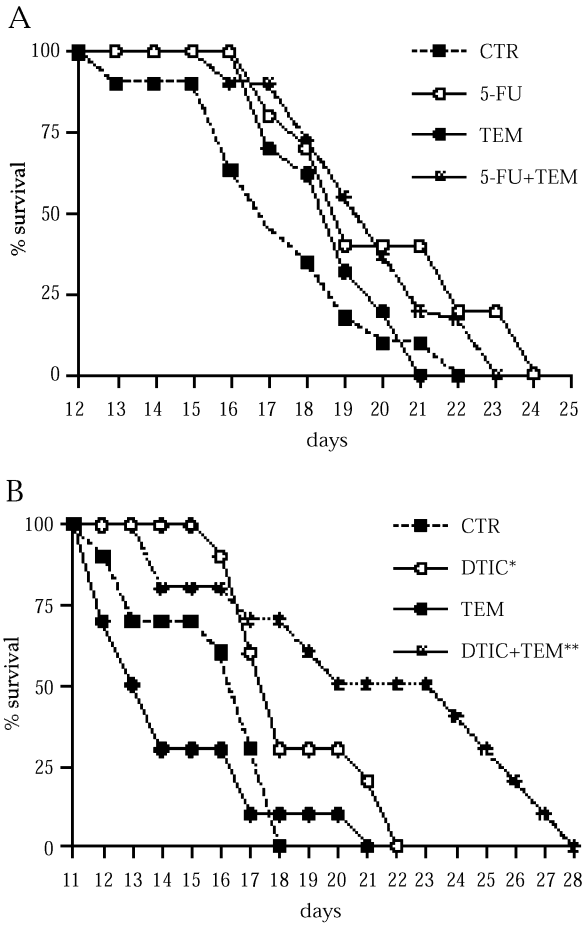

Figure 2. *Effect of trace element administration on the survival of mice treated with chemotherapy (3LL-HH surgical model).* ***(A)*** *5-FU and trace elements. Animals were treated with 5-FU (single dose of 50 mg/kg i.p. on the 7th postinoculation day) and/or trace elements (500-5000 μg/kg per os for 14 days). CTR= control, 5-FU and TEM are single treatments, 5-FU+TEM = combination. Data are expressed in % of surviving animals (n=/>10 per group).* ***(B)*** *DTIC and trace elements. Animals were treated with DTIC (60 mg/kg/day) and/or trace elements (5000 μg/kg/day) following the removal of the primary spleen tumor (day 3) for 14 days. Data are expressed in % of surviving animals (n=/>10). CTR= control, DTIC= dacarbazin treated group, TEM= trace element treated group, DTIC+TEM= combination treatment. *= p=0.067, **=p<0.05, Mantel-Cox method.*

Discussion

These studies further support the idea that certain trace elements have significant antitumor activity which can be exploited in local as well as in systemic therapy of tumors when dissemination of the disease occurs. This beneficial effect however is not enough to significantly extend the life-expectancy of the animals, most probably due to the rebound effect of the surviving tumor population so common in the human situation.

Since in clinical settings patients may expose themselves to trace elements during chemotherapy, we have analyzed in an experimental model the possible interaction of trace elements with chemotherapy. These studies indicated that trace elements may be able to promote the antitumor effects of certain commonly used chemotherapeutic agents as was found for DTIC in this murine lung carcinoma model. However, we have to mention that no such promoting effect of trace elements was found for 5-FU, although 3LL-HH cells are basically insensitive to this drug. Literature data indicate that trace elements differentially modulate the effects of chemotherapeutic agents depending on the target tissues. Effects of adriamycin (ADM) on host tissues was inhibited by bismuth with retention of the antitumoral effect. On the other hand Cu and Zn inhibited not only the side effects of ADM but the antitumoral one as well.[8] Zn was also shown to suspend the side effects of daunorubicin as well, mediated through induction of metallothionein expression.[9] In another study it was demonstrated that cisplatin uses copper export pumps where Cu can promote the development of resistance.[10] These data and our observation suggest a highly specific interaction of trace elements and various chemotherapeutic agents commonly used in cancer patients. These interactions are specific in respect of trace elements, the drug and the tumor type. Accordingly, the administration and combination of trace element supplement to a specific chemotherapy must be tested preclinically in relevant models before using in patients.

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