Effect of Tumour Regression on Serum and Tissue Copper Concentration in Mice Bearing Induced Fibrosarcoma

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Serum and tissue copper concentration was determined in mice bearing induced fibrosarcoma after successful chemotherapeutic treatment. The results showed a significant depression in copper concentration in the serum and tumour tissue after treatment with anti-cancer drugs. However, the liver copper concentration showed no significant change in treated groups of mice compared to before treatment.

Introduction

Elevation of copper in the serum of tumor-bearing animals as well as in humans with primary tumours of different localisation have been reported earlier [1, 2]. These results also reflected the usefulness of raised serum copper concentration in determining the activity of the disease. Therefore to study the importance of this measurement in prognosis, serum copper was determined in the tumour bearing mice both before and after treatment with chemotherapeutic drugs. It was carried out in animal tumour system first, because the clinical evidence of a therapeutic advantage established with animal tumours, can suitably be incorporated into clinical practice. To further investigate the correlation, the tissue copper concentration was also determined both before and after treatment with chemotherapeutic regimens.

Materials and Method

Establishment of tumour

All experiments were carried out in male strain A/RB mice.

The solid tumour was developed by the inoculation of 0.3 ml of 0.133 percent benzo(a)pyrene suspension in olive oil into the right thigh of 100 mice. Tumour growth was detected 3–4 months after inoculation in about 50 percent of the mice. Histological studies of the tumour tissue showed a fibrosarcomatous appearance.

Chemotherapy of tumour

(1) 5-Fluorouracil obtained from the Sigma Chemical Company was dissolved in physiological saline and injected in a dose of 25 mg/kg body weight for 7 consecutive days [3]. Ten normal and ten tumour bearing mice received the injection. After 3 to 4 days of the anticancer drug treatment, the tumour volume was measured using the formula $\Pi \times D_1 \times D_2 \times D_3$ where D_1, D_2, and D_3 denotes the length, breadth and height of the tumour (4). The blood was collected from the mice by slitting the jugular vein. The serum was separated and analyzed for copper by Atomic Absorption Spectrophotometer.

(2) 2 mg mitomycin C obtained from the Sigma Chemical Company was dissolved in 0.8 ml of methanol and then diluted to desired concentration with physiological saline. The solution was prepared on 1st day of the week and stored for use through-out the week. The dose given was 8.4 mg per kg body weight. Ten tumour bearing mice as well as ten normal strain A mice received the drug. The whole dose was given in two separate injections at intervals of 7 days. After treatment, when regression of tumour size was visible, the tumour volume was measured. The normal, untreated as well as treated mice were killed by slitting the jugular vein. From the blood collected serum was separated. The serum copper concentration was determined by Atomic Absorption Spectrophotometer in both control and tumour bearing animals. The liver as well as the tumour tissue were also dissected out from the mice homogenized in 0.25 M sucrose solution and the tissue copper was also estimated by Atomic Absorption Spectrophotometer. For control study of tumour tissue, homologous tissue from the same site was taken from normal mice.

Results

Table I shows the tumour volume before and after treatment with anti-cancer drugs. The table shows that there is significant regression in tumour volume following treatment with both mitomycin C and 5-fluorouracil. The regression in case of mitomycin C treated mice was 76 percent and in 5-fluorouracil

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treated mice it was 57 percent, compared to untreated condition.

In Table II, the serum copper concentration is shown both before and after treatment. The serum copper concentration which was raised as a result of tumour growth, was again depressed after successful treatment with both drugs. It was 50 percent in mitomycin treated mice and 32 percent in case of 5-fluorouracil treated mice, compared to their untreated condition.

Table III and Fig. 1 gives the tissue copper concentration before and after treatment with anticancer drugs. It is also observed that though the tumour had regressed to a great extent, there was little change in the copper concentration in liver of treated mice compared to untreated condition. However, in the tumour tissue a striking reduction in copper concentration was noticed. The tissue copper concentration which was greatly increased as a result of tumour formation, fell significantly after treatment suggesting a positive correlation with the tumour burden.

Table I. Tumour volume before and after treatment with chemotherapeutic drugs.

Treatment	Number of mice	Tumour volume (C.C.) Before treatment	After treatment
Mitomycin C	10	2.45 ± 0.8	0.6 ± 0.2*
5-Fluorouracil	10	3.0 ± 0.9	1.3 ± 0.4

Results are indicated as mean ± S.E.M.
* Indicate level of significance $P < 0.05$.

Table II. Serum copper concentration in mice bearing induced fibrosarcoma before and after treatment.

	Normal control (10)	Fibrosarcoma bearing mice (10)	5-FU treated mice (10)	Mitomycin-C treated mice (10)
Copper concentration (μg/dl)	84.8 ± 4.8	231.9 ± 6.9*	159.2 ± 10**	116 ± 3**

Units are quoted as mean ± S.E.M.
* Differences significant at $P < 0.05$.
** Differences significant at $P < 0.01$.

Table III. Copper concentration in liver and tumour tissue of mice bearing induced fibrosarcoma before and after treatment with mitomycin C.

Tissue	Normal Control (10)	Fibrosarcoma mice Before treatment (10)	After treatment (15)
Liver	14.6 ± 1.7	10.6 ± 1.1* (10)	10.2 ± 4 (7)
Tumour tissue	10.4 ± 5	26.2 ± 3.8* (10)	11.7 ± 2* (8)

Units are quoted as μg/gm of wet tissue.
Values are given as mean S.E.M.
The numerals in parentheses indicate the number of mice.
* Differences significant at $P < 0.01$.

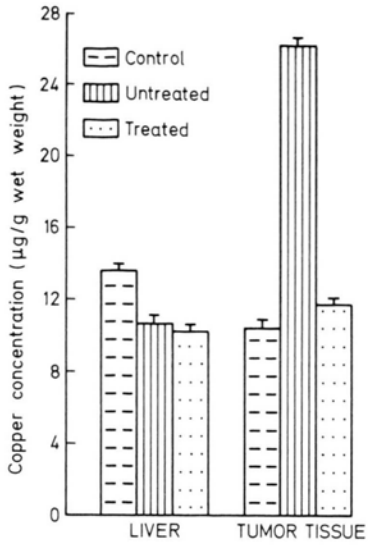

Fig. 1. Copper concentration in the liver and tumour tissue of control, tumour bearing and treated mice. The results are significant in the tumour tissue ($P < 0.05$).

Discussion

Earlier studies have revealed that the serum copper concentration is correlated with disease activity in human cancer patients and also in mice bearing different types of tumours [1, 2]. Interestingly, in this study the measurement of Serum copper in experimental mice made after successful treatment with anticancer drugs under controlled conditions revealed that along with tumour regression there was significant depression in serum copper concentration. Such results have been reported earlier in human patients with tumour of different sites [1].

The correlation of copper concentration with tumor regression was also well demonstrated by reduced copper concentration observed in tumour tissue after successful treatment. The tissue copper concentration which was elevated with the development of tumour, was again depressed almost to the normal range with reduction of the tumour volume. However, there was hardly any change in the copper concentration of the liver of tumour bearing mice after successful treatment with anti-cancer drugs. This seems to be due to the disturbance in the liver functions with the development of tumour [5].

Thus the serum copper concentration seems to have direct correlation with tumour regression, and it can be of substantial help in determining remission of the disease in mice bearing induced fibrisarcoma.

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