Significance of Zinc Metabolism in Blood Granulocytes in Surgical Cancer Patients

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INTRODUCTION

It has been suggested that the metabolism of zinc, one of the essential elements in human organism, undergoes characteristic changes in cancer patients. In general, cancer tissues contain high concentrations of zinc, while its content in serum and whole blood decreases significantly[1)17)21)22)].

Although neutrophilic and eosinophilic blood granulocytes contain the highest concentration of zinc[20)], this high values of zinc are found to decrease markedly in folic acid deficiency anemia and leukemias[4)22)]. Unfortunately, its biochemical determination is too complicated for clinical routine analysis.

In 1957, McNary[9)] offered a histochemical dithizone method for the detection of zinc in granulocytes in blood smears and bone marrow preparations. Using this simple method Szmigielski and Litwin[15)] confirmed decreased values of zinc in blood granulocytes in leukemias and other neoplastic diseases. Thereafter, Brunner and Frühwald[3)] observed that the decrease in zinc content occurred even at the early stage of some carcinomas. This histochemical method may be useful as a clinical routine method to elucidate zinc metabolism in blood granulocytes of cancer patients.

In the present study the zinc content in blood granulocytes of cancer patients and its changes following surgical operation and cancer chemotherapy were histochemically studied, and the clinical significances of zinc metabolism in blood granulocytes were examined in relation to the progress of cancer.

MATERIALS AND METHODS

The determination of zinc content in blood granulocytes was performed in 112 patients with various neoplastic diseases (56 with gastric carcinoma, 14 with carcinoma of the colon and rectum, 10 with carcinoma of the breast, 6 with carcinoma of the thyroid gland and 26 others) and 84 non-neoplastic patients.

The procedure of the histochemical method of McNary[9)] for the detection of

zinc in blood granulocytes is as follows: 1. Obtain peripheral blood by finger or ear puncture and prepare fresh air-dried blood smears. 2. Dip quickly 2 to 3 times in distilled water and drain. 3. Flood with staining solution which is consisted of 1.5 parts of 0.01 % dithizone in absolute aceton and 1 part of zinc-free water. 4. Stain until staining solution has changed from clear green to straw yellow. 5. Rinse in 3 changes of distilled water. 6. Stain the nucleus in 0.1 % aqueous azur II. Dithizone reactive material in granulocytes is stained as various numbers of golden or reddish brown granules (Fig. 1). The results were given using a score method according to Szmigielski and Litwin[15]; no dithizone-stained granules were defined as score 0, less than 10 granules as score 1, 10 to 30 granules as score 2, more than 30 granules as score 3, and numerous granules as score 4. The zinc content was represented by the total of each score in 100 granulocytes, designated as "zinc score".

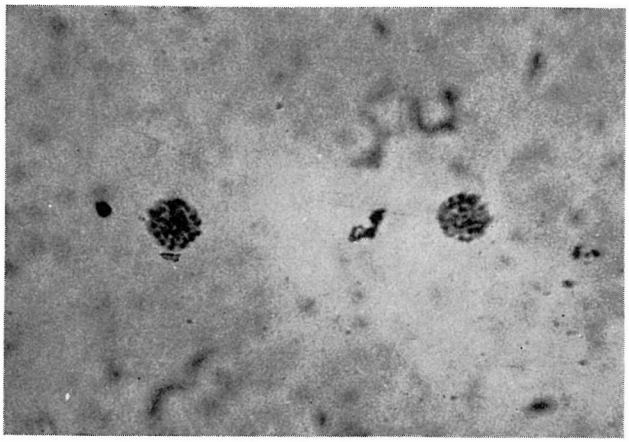

Fig. 1. Dithizone stained granules in blood granulocytes.

RESULTS

Zinc score in preoperative cancer patients

The zinc content of blood granulocytes in 112 patients with various neoplastic diseases is shown in Fig. 2. The zinc score of cancer patients was 102 ± 34, being markedly lower than a score of 158 ± 25 in 84 non-neoplastic diseases. Seventy (62.5 %) patients with neoplastic diseases had a score of less than 110, while only 2 (2.4 %) had such low score in non-neoplastic diseases. We consider that a score of less than 110 strongly suggests the existence of carcinomas and a score between 110 and 130 is borderline. However, it is noteworthy that the zinc score was not lowered in most of early carcinomas, i, e., small carcinomas without metastasis in the thyroid gland and breast or the carcinomas confined to

the mucosa and submucosa in the gastrointestinal tract.

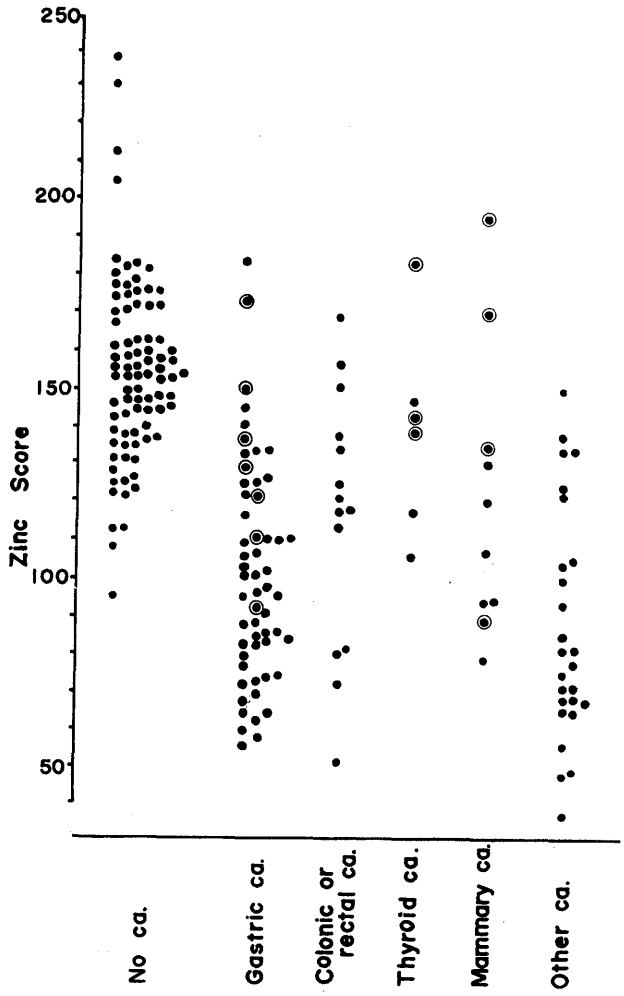

Fig. 2. Zinc score of cancer patients. ⊙ early carcinomas

The zinc score in relation to radical resectability of carcinomas

As shown in Fig. 3, the zinc score was less than 110 in 22 (91.7 %) of 24 patients with non-resectable carcinomas, while it varied between 51 and 196 in resectable carcinomas.

Changes in the zinc score following the curative operation

Without cancer chemotherapy or radiation therapy. After the curative operation, the zinc score of 10 patients with neoplastic diseases (3 with carcinoma of the colon, 3 with carcinoma of the rectum, 2 with gastric carcinoma, 1 with carcinoma

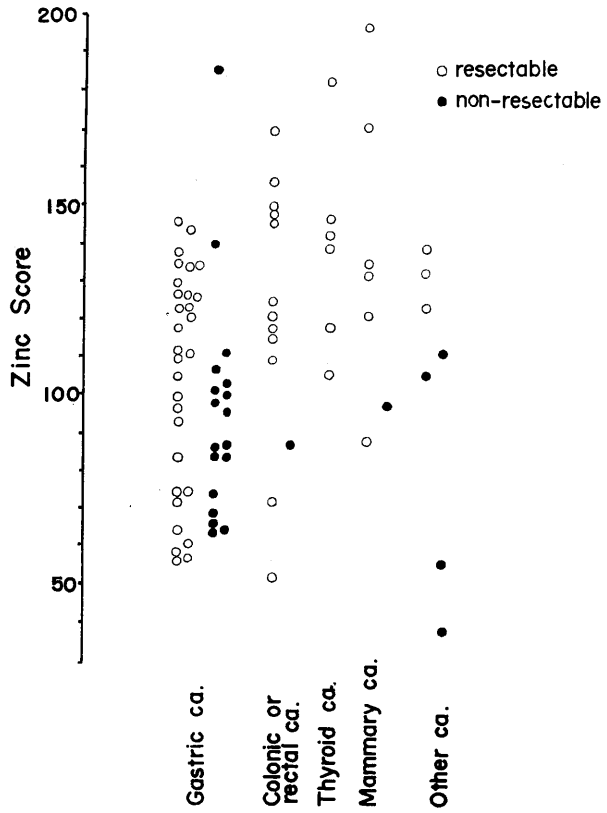

Fig. 3. Zinc score in relation to radical resectability of carcinomas.

of the thyroid gland and 1 with carcinoma of the breast) changed as shown in Fig. 4. In 5 cases with a preoperative score of less than 110, the score was elevated to normal values (more than 120) 3 to 5 weeks after the operation, although the changes were not significant in 2 weeks. In 4 cases with a preoperative score of more than 120, the score was, more or less, elevated postoperatively.

With cancer chemotherapy. Postoperative changes in the zinc score in 13 patients with gastric carcinoma are shown in Fig. 5. In each case chemotherapeutic agents (Mitomycin C and/or 5-fluorouracil) were administered for about 4 weeks after the operation. In 6 cases with a preoperative score of less than 110, the score was gradually elevated and reached to values of more than 120 in 4 to 5 weeks. In the most prominent case, the preoperative score of 55 was elevated to 117 in 2 weeks, 143 in 5 weeks and the high value was maintained thereafter. We have the impression that the improvement of the zinc score following the curative operation occurs somewhat earlier than that of humoral factors such as hemoglobin content, the ratio of albumin to globulin and lactic dehydrogenase activity in serum.

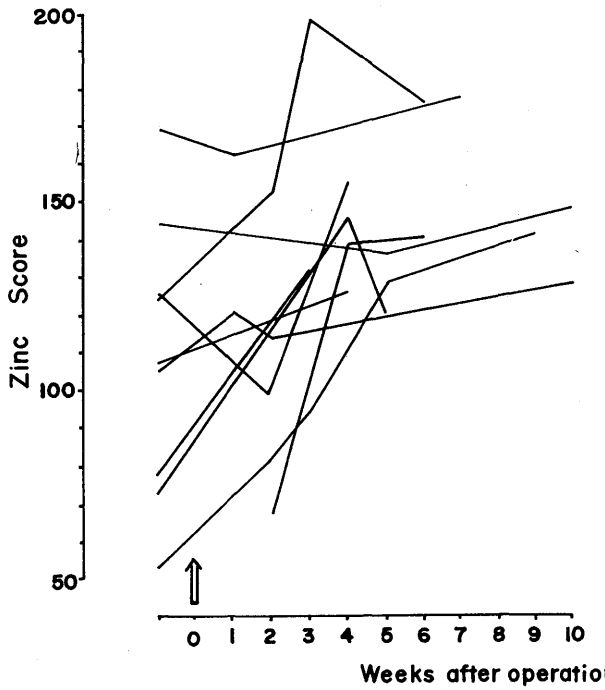

Fig. 4. Changes in zinc score following curative operation of carcinomas without cancer chemotherapy or radiation therapy.

Changes in the zinc score following non-curative or palliative operation

Changes in the zinc score in 7 patients with gastric carcinoma following non-curative or palliative operation are shown in Fig. 6. After the operation chemotherapeutic agents were administered as long as possible. In contrast with a distinct increase following the curative operation, the zinc score seemed to run parallel with changes in general conditions of cancer patients.

Changes in the zinc score following cancer chemotherapy in inoperable cancer patients

Changes in the zinc score following cancer chemotherapy were studied in 13 inoperable cancer patients (10 with gastric carcinoma, 2 with carcinoma of the breast and 1 with malignant parotid tumor). The effectiveness of cancer chemotherapy was judged on the basis of the criterion set by the committee of the Japan society for cancer therapy. Simultaneous radiation therapy was made in 2 carcinomas other than gastric carcinoma. As shown in Fig. 7, the zinc score was gradually elevated toward normal values in case of improvement. In case of aggravation the zinc score was lowered or unchanged when it was low in the beginning.

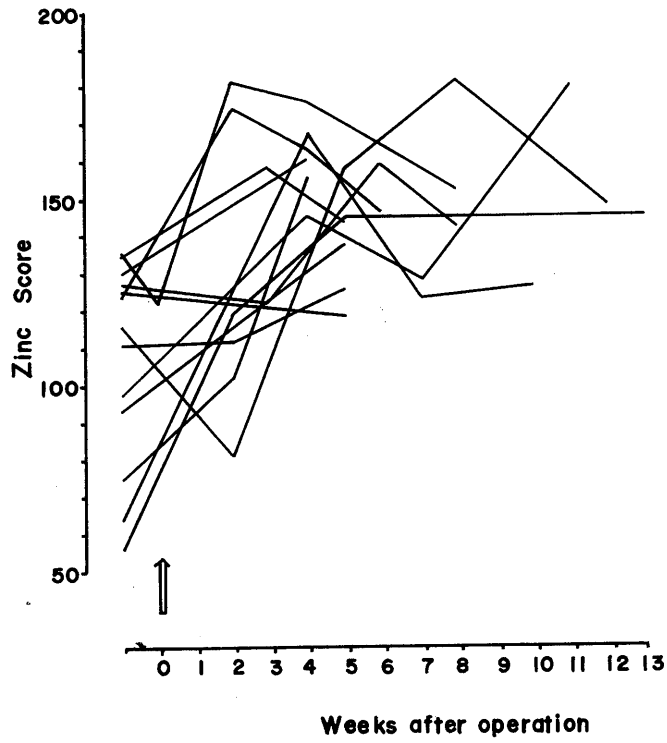

Fig. 5. Changes in zinc score following curative operation of gastric carcinomas with cancer chemotherapy. Mitomycin C and/or 5-fluorouracil were administered for about 4 weeks after operation.

DISCUSSION

In the present study, decreased values of zinc in blood granulocytes were obvious in cancer patients. However, the zinc score was generally not lowered at the early stage of carcinomas, in contrast with the observation of Brunner and Frühwald[3] in carcinoma of the pharynx, tongue and tonsil. Fujiwara[6], in our laboratory, showed in tumor-bearing mice that the incorporation of ^{65}Zn into blood corpuscles markedly decreased at the time when metastases of cancer occurred in the retroperitoneal lymphnodes and liver. Takano et al.[16] confirmed by atomic absorption spectroscopy that serum zinc level was not lowered in malignant diseases without recognizable metastases. These observations indicate that blood zinc level is not lowered until cancer progresses beyond a certain extent. Therefore, it seems reasonable that the zinc content of blood granulocytes, given using the score method, is not useful for the diagnosis of early carcinomas. At the advanced stage of carcinomas the zinc score was almost always lowered; 91.7 % of the cases had a score of less than 110. We predict preoperatively that radical operation may

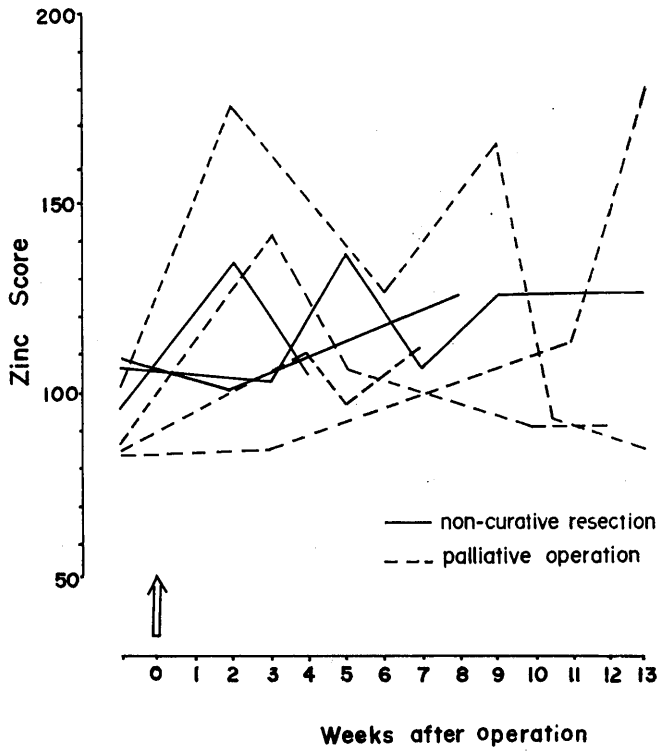

Fig. 6. Changes in zinc score following non-curative or palliative operation of gastric carcinomas with cancer chemotherapy.

be successfully performed in cancer patients with a score of more than 110.

It has been reported that cancer tissues have a higher content of zinc and the zinc content of whole blood of cancer patients is subnormal, unless the neoplasms develop in tissues relatively rich in zinc —— such as the prostate gland and bone[1)12)17)]. Some investigators[6)8)10)18)] have shown in tumor-bearing animals that the incorporation of ^{65}Zn increases in cancer tissues and the liver, while it decreases in blood corpuscles and serum. Since the whole-body retention of zinc has an increasing tendency in tumor-bearing animals[19)], it seems unquestionable that the transfer and accumulation of zinc in cancer tissues and the liver takes some responsibility for decreased values of zinc in blood granulocytes of cancer patients. On the other hand, Shiraishi et al.[13)] showed that the ^{65}Zn uptake of blood granulocytes of cancer patients was significantly lower than that of non-cancer patients after the intravenous injection of ^{65}Zn, while there was no difference between the two after the addition of ^{65}Zn to the incubation medium containing blood granulocytes. From these observations they have assumed that decreased values of zinc in blood granulocytes of cancer patients are caused by a decrease

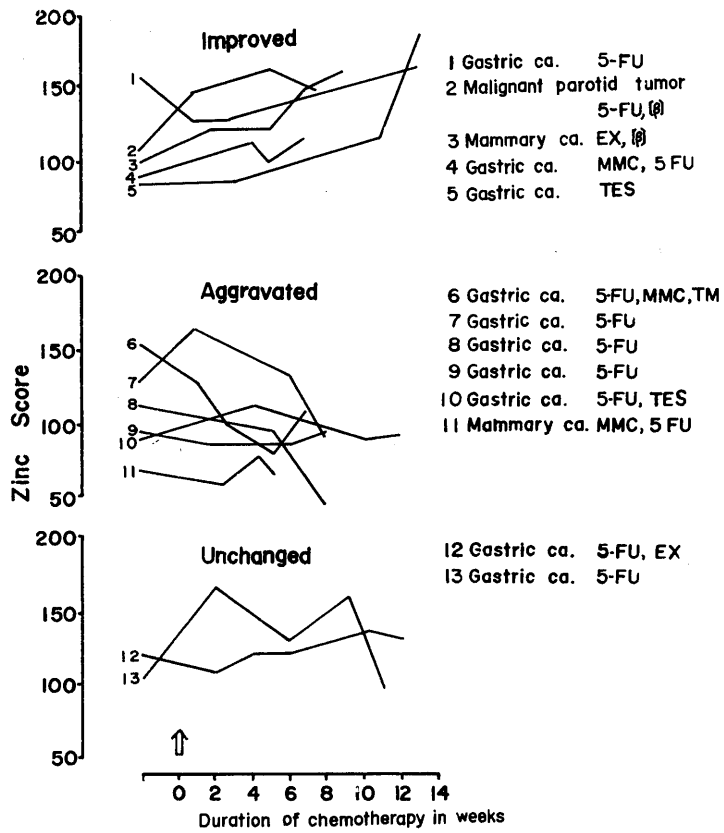

Fig. 7. Changes in zinc score following cancer chemotherapy in inoperable cancer patients. 5-FU = 5-fluorouracil EX = Endoxan TES = Tespamin MMC = Mitomycin C TM = Toyomycin [β] = radiationtherapy

of zinc incorporation in the maturation process in the bone marrow. Recently Nieburgs and Goldberg[11] showed that the nuclei of polymorphonuclear leukocytes of cancer patients underwent morphological alterations designated as "malignancy associated changes", suggesting systemic involvement in neoplastic diseases. It is likely that changes in zinc metabolism in blood granulocytes of cancer patients are a manifestation of systemic disorders in metabolism. Thus, the elevation of the zinc score following the curative operation is probably due to both the abolition of much accumulation of zinc in cancer tissues and the improvement of systemic disorders in metabolism.

The elevation of the zinc score following the curative operation and effective cancer chemotherapy and its lowering in advanced carcinomas suggest that zinc metabolism in blood granulocytes is greatly affected by the progress of cancer. Gibson et al.[7] observed that the zinc content of leukocytes was markedly lowered

in leukemias and these low values returned to normal by successful therapy. Similar observation was made by Wolff[22]. Moreover, Addink and Frank[2] showed that the low zinc level of whole blood in various neoplastic diseases increased to normal values in case of favorable progress, while it was persistent or decreased still further in case of unfavorable progress. In view of their observations it may be concluded that there exists a close relation between the zinc content of blood granulocytes and the progress of cancer; when cancer tissues are surgically removed or tumor growth is suppressed by cancer chemotherapy and others, the zinc score is elevated. On the contrary, when tumor growth is accelerated and the systemic effects of cancer are aggravated, the zinc score is lowered. Thus, the histochemical determination of the zinc content of blood granulocytes can be applied to examine the postoperative courses and th effectiveness of cancer chemotherapy.

SUMMARY

Significance of zinc metabolism in blood granulocytes was studied in surgical cancer patients. The zinc content of blood granulocytes was analyzed using the histochemical method of McNary and the score method of Szmigielski and Litwin. The zinc score in preoperative cancer patients was markedly lower than that in non-cancer patients. This low values were not observed in most of early carcinomas while the score was almost always lowered in non-resectable carcinomas. This suggests that zinc metabolism in blood granulocytes is not disturbed until cancer progresses beyond a certain extent. The low score in preoperative cancer patients was elevated to normal values 3 to 5 weeks after the curative operation with or without cancer chemotherapy. In non-resectable carcinomas, changes in the zinc score following cancer chemotherapy seemed to run parallel with its effectiveness. These observations indicate that there is a close relation between the zinc content of blood granulocytes and the progress of cancer. This histochemical method is useful as a clinical routine method to examine the progress of cancer.

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