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Thiamin Status of Patients Treated with Drug Combinations Containing 5-Fluorouracil

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Abstract—*Thiamin status has been assessed from whole blood transketolase measurements in patients with cancer in various sites being treated with drug combinations which included 5-fluorouracil (5-FU). Longitudinal measurements in nine patients showed that thiamin deficiency developed during treatment with 5-FU and was corrected by giving* 100 mg *thiamin per* day. *Similar evidence of thiamin deficiency was found in* 26 *patients who had been treated with 5-FU-containing regimens for varying periods of time but was not found in patients treated with regimens not containing 5-FU. Giving high doses of thiamin reduced the high levels of plasma and urinary ribonuclease which was of hepatic origin.*

INTRODUCTION

CACHEXIA is a feature of many patients with malignant disease [1] and is associated with a poor intake of all nutrients. It is increasingly being recognised, however, that the cancer patient may develop a deficiency of a specific nutrient and that the nature of the nutrient may vary with different kinds of tumour [2]. Thiamin plays a vital role in energy metabolism. Biochemical evidence of thiamin deficiency, as determined by the thiamin pyrophosphate (TPP) stimulating effect of red cell transketolase activity [3, 4] has been reported in patients with cancer [5, 6]. Since some of these patients were receiving a vitamin supplement it seemed unlikely that this deficiency was simply due to poor dietary intake [5]. Patients with breast cancer have been found to have higher TPP values than either patients with cancer in other sites or patients with non-malignant but debilitating diseases. There is also some evidence that the degree of thiamin deficiency increases with the progress of breast cancer [7].

The cytotoxic drug, 5-fluorouracil (5-FU), is widely used either alone or in combination, in the treatment of disseminated cancer and particularly of breast cancer [8]. Studies on cancer patients have indicated that the thiamin deficiency associated with the presence of a tumour is exacerbated by treatment with the drug [6, 9]. Furthermore, experimental studies have shown that treatment of rats with high (45 mg/kg body wt) or low (7.5 mg/kg body wt) doses of 5-FU decreases the concentration of thiamin in the liver and spleen and increases the TPP value in whole blood [9]. These results are also parallel to those of *in vitro* studies [5, 9].

The present study was undertaken to investigate the longitudinal development of thiamin deficiency in patients during treatment with 5-FU, and to study the effect in such patients of thiamin supplementation. Since protein-energy malnutrition (P.E.M.) commonly occurs in patients with cancer, opportunity has been taken in the present study to determine urinary and plasma ribonuclease activity, since these have been used as a sensitive index of P.E.M. [10].

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MATERIALS AND METHODS

A total of 35 patients bearing tumours in various sites, including breast (29), lung (1), bronchus (1), sigmoid colon (1), parotid (1), rectum (1) and adrenal (1) were studied. These patients were divided into two groups. Group I contained nine patients with a mean age of 46.7 yr (range 30–59 yr) who had had no prior treatment and were studied from the commencement of chemotherapy. After collecting five heparinized blood samples at various intervals from each of these patients, they were given a supplement of thiamin-hydrochloride (100 mg/day). Group II contained 26 patients with a mean age of 61.6 yr (range 41–75 yr) who had already received chemotherapy for 2–12 months before they were included in the study.

All patients had undergone radiotherapy and surgery. They were given either 5-FU alone (500–750 mg) or in combination with other drugs, such as cyclophosphamide (300–500 mg), vincristine (0.5–1 mg), methotrexate (20–50 mg), and metoclopramide (10 mg), for either 2 or 5 days per month. the results on these patients were compared with 10 age–sex-matched healthy volunteers and 10 age–sex-matched cancer patients who were being treated with drugs other than 5-FU.

Blood samples were collected from the antecubital vein into heparinized tubes for the determination of transketolase activity, and into EDTA tubes for the determination of plasma ribonuclease activity. The first urine sample voided after the blood sample had been taken was collected into a plain container. The samples were placed in a deep freeze (−40°C) until analysed. The haemolyzed whole blood was used to measure the activity of transketolase by a simplified method [11]. The enhancement of enzyme activity resulting from the addition of thiamin pyrophosphate (TPP) was expressed as a percentage of the original activity and is referred to as the "TPP stimulation" or "TPP effect". Values obtained without the addition of thiamin pyrophosphate represented the absolute enzyme activity [4]. The modified thiochrome method [12] was used for the determination of thiamin in urine. Free alkaline ribonuclease activity was measured in plasma and urine by a modification of the method of Roth [13]. The change in absorbance of the samples was ultimately expressed as units per ml (U/ml). The alkaline picrate method [14] was employed for the determination of urinary creatinine. The significance of differences between the experimental and control groups was tested using Student's *t*-test.

RESULTS

The TPP stimulating effect of the transketolase activity (TK) in the whole blood of the patients before undergoing chemotherapy (Table 1) was within the normal range (<15% [15]) and rose significantly ($P<0.001$) when the patients were treated with 5-FU for one month in combination with other cytotoxic drugs. The urinary value of thiamin did not change significantly during this phase of treatment. However, after giving thiamin supplements (100 mg/day) for 4–12 weeks, the excretion of thiamin rose ($P<0.01$) and this rise was accompanied by a fall in the TPP value ($P<0.001$) to a value which was not significantly different from that prior to receiving chemotherapy.

Treatment with cytotoxic drugs, other than 5-FU had no significant effect on either the TPP value or urinary thiamin excretion when compared with age-matched healthy control subjects (Table 2). When the drug regimen contained 5-FU, however, the TPP effect was significantly higher ($P<0.001$) and the urinary thiamin significantly lower ($P<0.001$) than when it did not. The values in these patients also differed with the same degree of significance from the healthy controls.

The plasma and urinary free alkaline RNase activity of the patients before chemotherapy whose thiamin results are given in Table 1 were higher than those of the healthy control subjects and did not change after treatment for 8–20 weeks with 5-FU (Table 3). However, both plasma and urinary RNase activities fell significantly when the patients being treated with 5-FU were given thiamin.

DISCUSSION

Two groups of patients treated with 5-FU were studied. In one of these, the patients had been treated for 2–12 months with combination chemotherapy and these patients had biochemical evidence of thiamin deficiency. This deficiency was shown to be due to 5-FU since patients treated with a combination of various cytotoxic drugs excluding 5-FU had a similar thiamin status to those of age–sex-matched healthy controls. Thus, these observations confirm those of our previous study [5] and those of others [6]. The other group of patients treated with 5-FU was studied

Table 1. The effect of treatment with 5-fluorouracil (5-FU) on the "TPP effect" of whole blood and the urinary excretion of thiamin in patients with cancer

	Pre-chemotherapy	Post-chemotherapy	Post-chemotherapy + thiamin
TPP effect%	8.5±2.4	31.4±2.7*	10.5±2.6†
Urinary Thiamin μg/g creatinine	72±23	50±11	316±80‡

Significance of difference between initial values and those after chemotherapy; $*P<0.001$.
Significance of difference between values after chemotherapy alone, and after chemotherapy + thiamin: $\dagger P<0.001$; $\ddagger P<0.01$.
Values are the mean of the five values ±S.E.M., obtained for nine patients studied longitudinally.

Table 2. The effect of treatment with 5-FU on the "TPP effect" of whole blood and the urinary excretion of thiamin in patients with cancer and in age–sex-matched healthy control subjects

Group	Number of subjects	TPP effect %	Urinary thiamin μg/g creatinine
Treated with 5-FU*	26	20.2±2.0†‡	49±7†‡
Treated with other cytotoxic drugs only	10	11.0±1.3	119±19
Healthy control	10	7.8±1.7	93±8

*Patients given 5-FU in combination with other cytotoxic drugs.
Significance of difference between values of patients treated with 5-FU and those healthy controls: $\dagger P<0.001$.
Significance of difference between values of patients treated with 5-FU and those treated with other cytotoxic drugs: $\ddagger P<0.001$.
Values are mean ±S.E.M. for the number of patients or subjects shown.

Table 3. Plasma and urinary ribonuclease activity in patients before and after treatment with 5-FU and after supplementation with thiamin and in healthy control subjects of similar age

	Healthy subjects	Patients Pre-chemotherapy	Patients Post-chemotherapy	Patients Post-chemotherapy + thiamin
Number of subjects	10	9	32	9
Plasma RNase (Unit/ml)	2624±161	4655±323*	4404±193‡§	3136±294‖
Urinary RNase (Unit/ml)	4293±694	9610±1731†	9148±892§	4866±699 ¶

Significance of difference between values of pre-chemotherapy and post-chemotherapy: $\dagger P<0.02$; $*P<0.01$.
Significance of difference between values of post-chemotherapy and those healthy controls: $P<0.01$; §<0.001.
Significance of difference between values of post-chemotherapy and post-chemotherapy + thiamin shown: ¶ $P<0.02$; ‖ $P<0.01$.

longitudinally. These patients had no evidence of thiamin deficiency before receiving 5-FU, but the first blood sample taken after receiving the drug for 1 month showed an elevated TPP value in all of nine patients. The treatment with 5-FU did not precipitate any side effects such as nausea, vomiting and diarrhoea in the patients. Furthermore, the appetite of the patients was similar to that of the patients treated with cytotoxic drugs other than 5-FU. It is, therefore, unlikely that the biochemical evidence of 5-FU-induced thiamin deficiency was the consequence of reduced dietary intake.

The raised values for the TPP effect as a result of 5-FU treatment were reversed by giving a large dose of thiamin (100 mg/day) daily. This treatment with thiamin had two potentially important consequences. Four patients reported that they felt better after receiving the vitamin and that their appetite improved. Moreover, plasma and urinary RNase concentrations which had been elevated before and during treatment with the drug returned to values statistically indistinguisable from those of healthy control subjects. Elevated values such as those found in the cancer patients before and during treatment are characteristic of protein-energy malnutrition and specifically are said to result from damage to the pancreas or liver. The ribonucleases from these two sites can be distinguished by their optimum pH's. In this study the enzyme activity was measured at pH 7.8 which is the optimum pH of liver RNase [16]. Furthermore, the activity of the enzyme was not affected significantly by the addition of copper ions to the medium (Table 4) [16], another characteristic which distinguishes the liver enzyme from that of the pancreas.

The results of this study suggest that patients with metastatic breast cancer have a leakage of RNase from the liver and that this process is prevented by giving high doses of thiamin. Biochemical changes in the liver of human patients with cancer [17] and of experimental animals injected with cancer cells [18] have been described when there is no evidence of metastatic disease. The raised levels of hepatic RNase in the plasma and urine of our patients may be further evidence of metabolic disturbances in an organ distant from the site of the tumour. It seems that this metabolic effect is rectified by giving thiamin.

The interaction of 5-FU with thiamin clearly merits further investigation. In our previous paper [5] the results of *in vitro* studies suggested that 5-FU prevented the phosphorylation of thiamin which is necessary to convert the vitamin into its active form, thiamin pyrophosphate. This could be explained on the basis of competition for phosphate by the drug which is converted first to 5-fluorouridine (FUR) and then to 5-fluorouridine 5-monophosphate (FURP) and 5-fluoro-2-deoxyuridine monophosphate (FUDRP). This is the active form of the drug and blocks the enzyme thymidylate synthetase [19]. Alternatively, it may be that the conversion of 5-FU to its deoxyriboside derivative requires thiamin pyrophosphate or actually increases breakdown of thiamin to its catabolic products. Furthermore, it would be of interest to ascertain whether the change in TPP effect correlates with the anti-tumour effect of 5-FU. Unfortunately the tumour response to the cytotoxic drug was not recorded in this investigation. Further studies are being instigated along these lines.

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Table 4. Plasma and urinary RNase activity, determined with and without addition of cupric chloride ($CuCl_2$; 1 mM) *in patients treated with 5-fluorouracil, before and after administration of thiamin*

	Without $CuCl_2$		With $CuCl_2$	
	Before thiamin	After thiamin	Before thiamin	After thiamin
Plasma	4979 ± 445	3087 ± 432*	5029 ± 484	3146 ± 254*
Urine	8894 ± 1236	4942 ± 683*	8709 ± 1181	5087 ± 585*

*Difference between values before and after thiamin significantly different ($P < 0.01$).
Each result is the mean ± S.E.M. of 17 samples obtained from nine patients.

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