Eur J Cancer Clin Oncol, Vol. 22, No. 7, pp. 837–842, 1986.
Printed in Great Britain.

0277-5379/86$3.00+0.00
Pergamon Journals Ltd.

Erythrocyte Polyamine Levels during Intravenous Feeding of Patients with Colorectal Carcinoma*

DAVID M. OTA,†‡ KENJI NISHIOKA,§ BRUCE GROSSIE‡ and DENNIS DIXON‖

Departments of ‡General Surgery, §Biochemistry and Molecular Biology and ‖Biomathematics, The University of Texas M.D. Anderson Hospital and Tumor Institute at Houston, Houston, TX, U.S.A.

Abstract—*Changes in erythrocyte (RBC) polyamines were studied during total parenteral nutrition (TPN) in 16 colorectal carcinoma patients and six patients with noncancerous diseases. RBC putrescine (PTC), spermidine (SPD), and spermine (SPM) were analyzed before and at the completion of preoperative TPN in each patient. Before TPN, nutritional status, based on a history of weight loss and plasma albumin, prealbumin and retinol-binding protein levels, were similar for the two groups. Initial RBC PTC, SPD, and SPM levels were also similar for both groups. Preoperative TPN induced significant increases in RBC PTC levels ($P<0.001$) and RBC PTC:SPD ratios ($P < 0.005$) of the cancer patients while no significant polyamine changes were observed in the other group. Host response to nutritional therapy was monitored with plasma prealbumin and retinol-binding protein levels which did not change significantly in either group.*

INTRODUCTION

ALTHOUGH considerable clinical data suggest that total parenteral nutrition (TPN) does not induce tumor growth in man [1], animal studies have shown that tumor proliferation may occur with aggressive forced-feeding methods [2–5]. Because physical examination or roentgenographic studies may not be sensitive enough to detect changes in tumor growth during short periods of TPN, more sensitive biochemical markers of tumor proliferation are needed to detect tumor growth in patients undergoing nutritional therapy.

Polyamines are ubiquitous, low molecular weight amines that are associated with protein, RNA, and DNA syntheses and their biosyntheses are essential for cell proliferation [6,7]. Elevated polyamines levels in urine, plasma, cerebrospinal fluid, and erythrocytes (RBC) of cancer patients have been reported and such observations are consistent with the uncontrolled growth behavior of neoplastic diseases [8–13]. The correlation of the thymidine labelling index of multiple myeloma cells with the urinary excretion of putrescine [14] suggests that polyamines may be sensitive markers of tumor proliferation during TPN.

Our study was designed to determine changes in RBC polyamine levels during preoperative TPN. In order to assess if RBC polyamines were derived from neoplastic tissue, RBC polyamines levels during TPN were measured in patients with benign disease and with colorectal carcinoma. Our results show that TPN is associated with increased RBC polyamines levels in colorectal carcinoma patients and not in noncancer patients.

MATERIALS AND METHODS

Patient population

Six noncancer patients and 16 colorectal carcinoma patients who required preoperative TPN to restore or prevent nutritional deficits before undergoing operative procedures were entered into this study. Patients who had received chemotherapy, radiotherapy or had undergone a surgical procedure within 3 months of this study were excluded. No patients received antineoplastic therapy during the study. All patients had normal kidney function (plasma creatinine less than 1.4 mg/dl and blood urea nitrogen less than 25 mg/dl) and normal liver function (serum bilirubin less than 1.5 mg/dl) during the study. Venous blood (7 ml) was collected in heparin-coated tubes before TPN began and again after nutritional therapy was completed before a planned surgical procedure. The second blood sample was obtained the day before the scheduled operative procedure when

Accepted 5 December 1985.
*This work was supported by grant RO1 CA 34465 from the National Cancer Institute.
†To whom correspondence should be addressed at 6723 Bertner Avenue, Houston, TX 77030, U.S.A.

TPN was still being administered. This study was conducted with approval of our institution's Human Surveillance Committee and with the consent of the patients.

Dukes' stage of malignant disease was documented by pathologic evaluation of the resected specimens and intraabdominal findings by the surgeons. For the noncancer group, diagnosis of benign disease was documented by exploratory laparotomy, histologic evaluation of the resected specimen, and length of disease-free survival since cancer therapy was completed (greater than 5 yr). Patients who were not metabolically stable or who had infections were excluded from both study groups. Furthermore, patients who did not have conclusive diagnoses of benign or malignant disease by pathologic verification were excluded from the study.

Nutritional regimens

Patients in both groups received a 25% glucose and 5% crystalline amino acid solution with 40–60 mEq/l NaCl, 20–40 mEq/L KCl, 10 mEq/L $MgSO_4$, 4.5 mEq/L calcium gluconate, 10–15 mEq/L KH_2PO_4, 2 ml trace elements, and MVI-12^R. TPN solutions were delivered through a central venous catheter and administered continuously over 24 hr at a constant rate of 40–60 calories/kg of body wt/d and 2–3 g crystalline amino acid/kg of body wt/d.

Evaluation of nutritional status and response to TPN

Nutritional status before and after preoperative TPN was determined in each patient by history of weight loss based on usual body weight and plasma visceral protein levels. Plasma albumin level (normal, 3.4–4.5 g/dl) was determined by bromcresol-dye assay [15]. Plasma prealbumin (normal, 10–40 μ g/dl) and retinol-binding protein (normal, 3–6 μg/dl) levels were determined with radial immunodiffusion plates (Calbiochem-Behring, La Jolla, CA). Per cent body weight loss and duration of TPN were compared for the two groups by using Student's *t*-test (two-tailed).

Polyamine analysis

RBC putrescine, spermidine and spermine levels were determined on a Durrum-Dionex D-500 amino acid analyzer (Dionex Corporation, Palo Alto, CA) by previously described methods [12]. Recovery of putrescine, spermidine, and spermine was 85%, 78.4% and 86.4%, respectively, and all values were corrected according to these percentages of recovery. The method used to analyze polyamine levels as low as 15 pmol had a coefficient of variation of less than 4% for within-run variation and of less than 7% for between-run variation. Changes in polyamine levels were determined for each patient by comparing RBC polyamine levels before and after preoperative TPN. Changes in RBC polyamine levels during TPN were statistically analyzed in each group by the paired Wilcoxon test.

RESULTS

Patient population

The clinical diagnoses and stages of disease for the two groups are shown in Table 1. In the noncancer group which included one male and five females, two patients were studied twice, receiving preoperative TPN 7 and 12 months apart and accounting for eight determinations of sequential RBC polyamine levels during TPN in this group. There were nine men and seven women in the colorectal cancer group. Women in both groups were anovulatory because of previous oophorectomy or age. Mean age for the colorectal cancer group was 70 ± 15 yr (mean ± S.D.) and 48 ± 22 yr for noncancer group. Preoperative TPN was given without infectious complications.

Nutritional status before TPN

Nutritional status before TPN was started is summarized in Table 2. To assess plasma visceral protein levels, the number of below-normal values of each protein was recorded for each patient. If all three plasma protein levels were normal, the patient was listed in column 3/3. Patients who had only two normal protein levels were placed in column 2/3. Assignments to columns 1/3 or 0/3 were made correspondingly. Weight loss and plasma visceral protein measurements were similar for the two groups.

Initial RBC polyamine levels

RBC polyamine levels and the ratios of putrescine:spermidine and spermidine:spermine before TPN was started are shown in Table 3.

Table 1. *Patient population*

Group	No. of patients
Colorectal cancer (n = 16)	
Stage	
B	2
C	8
D	6
Noncancer (n = 6)	
Benign gastric ulcer	2
Pseudointestinal obstruction	1
Radiation enteritis	1*
Short gut syndrome	1*
Benign oesophageal stricture	1

* Patients were studied twice for a total of eight sequential polyamine determinations for the noncancer group.

Table 2. Evaluation of nutritional status before TPN

Group*	Wt loss†	No. of patients with plasma visceral proteins below normal			
		o/3‡	1/3‡	2/3‡	3/3‡
Colorectal cancer (n = 16)	11.8 ± 4.9%	9	4	2	1
Noncancer (n = 8)	9.0 ± 4.1%	5	3	0	0

* n = number of determinations
† Based upon history of usual body wt, mean ± S.D.
‡ Number of below-normal plasma visceral protein levels (albumin, prealbumin, and retinol-binding protein) before TPN.

Host response to nutritional therapy

Duration of preoperative TPN was 8.4 ± 5.7 days (range: 4–28 days) for the colorectal cancer group and 8.4 ± 2.9 days (range: 7–15 days) for the noncancer group. Patients with colorectal carcinoma received 55 ± 11 cal/kg body wt/d and 2.6 ± 0.5 g crystalline amino acid/kg body wt/d; the noncancer group received 54 ± 12 cal/kg body wt/d and 2.7 ± 0.4 g crystalline amino acid/kg body wt/d. There was no significant difference in length of TPN or nutritional intake. By the paired t-test, no significant changes in plasma prealbumin and retinol binding protein levels were observed in either group (Fig. 1).

RBC polyamine levels during TPN

Significant increases in RBC putrescine levels and putrescine:spermidine ratios occurred during TPN in the colorectal carcinoma group (Fig. 2). There was a trend toward higher RBC spermidine levels during TPN, although not significant. RBC spermine level and spermidine:spermine ratios did not change during TPN. RBC polyamine levels or ratios did not change significantly during TPN in the noncancer group. Increased RBC putrescine levels and putrescine:spermidine ratios during TPN were unrelated to Dukes' stages B, C and D.

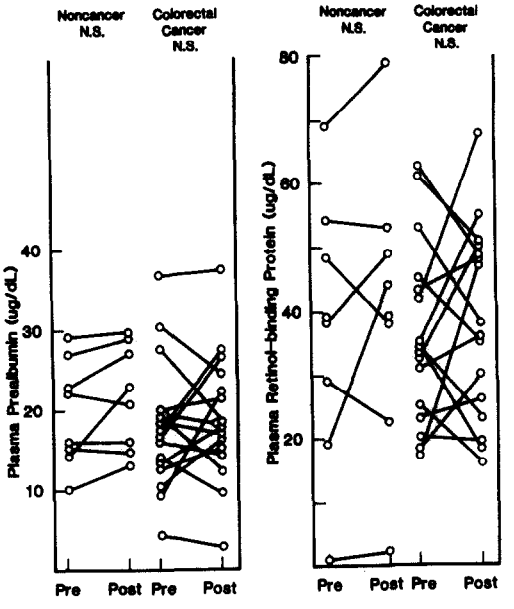

Fig. 1. Changes in plasma prealbumin (normal, 0–40 μg/dl) and retinol-binding protein (normal, 3–6 μg/dl) levels in noncancer and colorectal cancer patients during preoperative TPN. 'Pre' indicates plasma visceral protein levels before preoperative TPN; 'post' indicates levels after 7–10 days of preoperative TPN, but before surgery. N.S., not significant.

DISCUSSION

The possibility that administration of TPN solutions may promote tumor proliferation has been considered but never confirmed. Experimental studies provide conflicting results regarding tumor proliferation following host refeeding. Several investigators have reported enhanced tumor growth with increased host nutritional intake while others have not [2–5, 16–18]. Furthermore, extensive clinical observations, based on physical examination or roentgenographic studies, have not detected changes in tumor size with TPN [2].

Ambiguities regarding nutrition-induced increases in tumor growth may be partly explained by the growth pattern of solid tumors, which, based on a Gompertzian function, increases exponentially at first, then decreases and reaches a plateau [19]. By the time many tumors have been detected clinically, they have reached the plateau

Table 3. RBC polyamine levels before TPN

Group*	PTC†	SPD†	SPM†	PTC:SPD†‡	SPD:SPM†
Colorectal cancer (n = 16)	0.125± 0.094	20.46± 12.03	19.27± 16.23	6.23± 3.81	1.68± 1.26
Noncancer (n = 8)	0.191± 0.164	11.26± 5.14	7.43± 3.36	16.58± 13.84	1.65± 0.63

* n = number of determinations.
† PTC = putrescine, SPD = spermidine, and SPM = spermine. Values indicate mean nmol/ml packed RBC or ratio ± S.D.
‡ $\times 10^{-3}$.

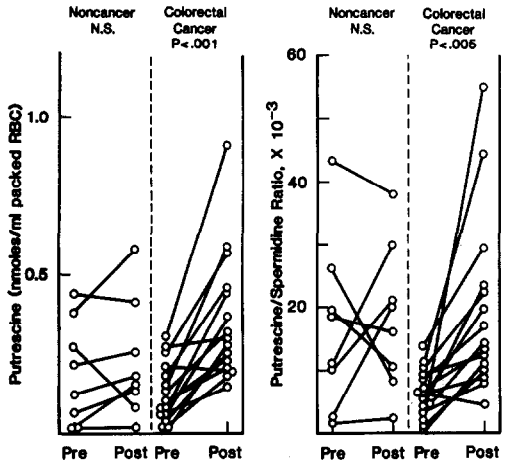

Fig. 2. Changes in RBC putrescine levels and putrescine:spermidine ratios in noncancer and colorectal cancer patients during preoperative TPN. 'Pre' indicates polyamine analysis before preoperative TPN; 'post' indicates levels after 7–10 days *of preoperative TPN, but before surgery. N.S., not significant.*

phase of the Gompertzian curve such that any change in growth during TPN may be small and undetectable. Therefore, if neoplastic proliferation is induced by TPN, sensitive markers of tumor growth must be developed.

The association of high ornithine decarboxylase (ODC) activity and putrescine production with cell proliferation indicates that polyamine synthesis has a regulatory role in cell growth. An important distinction of polyamine metabolism is that, compared with normal tissue, neoplastic tissue may have a greater requirement for polyamine synthesis [20–22]. Russell *et al.* [14] suggested that putrescine levels were related to the proliferative behavior of tumors, based upon the close correlation of urinary putrescine excretion and the thymidine labelling index of multiple myeloma cells.

Our study examined RBC polyamine levels based on a previous report showing that changes in RBC polyamines reflected the presence of malignant disease more accurately than do changes in plasma [12]. RBC contain approximately 80–90% of circulating polyamines; because they do not possess the enzymes to synthesize polyamines, they may be involved in polyamine transport from sites of production to organs of conjugation and excretion. In cancer patients, polyamines produced by tumor cells may be absorbed by RBC to maintain polyamine levels in various tissue and fluid compartments.

Patients were entered into this study based on several considerations. Patients receiving chemotherapy, radiotherapy or surgery were excluded because of potential changes in polyamine metabolism induced by these forms of therapy [11,23,24]. Women with active ovulatory cycles and patients with intercurrent infections were also excluded. Noncancer patients were verified to be free of malignant disease by the measures described. Furthermore, patients were studied only during preoperative TPN in which RBC polyamine levels were determined before and after completion of preoperative TPN. Although the cancer patients were significantly older than the noncancer patients, the pattern of blood polyamines in adults has been shown to be similar for this age group [25]. Sufficient data were not available to determine if age and sex were factors contributing to the variation of RBC putrescine levels in either group. This study was limited by the availability of only six noncancer patients who met the entry criteria for this group. Eight sequential polyamine determinations in the noncancer group may not be enough to be confident that there was no change.

One major question raised by this study is the tissue origin of increased RBC PTC levels during TPN in the cancer group. In an earlier study of TPN-induced changes in RBC polyamine levels, the cancer group had greater nutritional deficits, based on weight loss and plasma visceral protein levels compared with those of the noncancer group [26]. In the present study, the colorectal carcinoma patients were shown to have nutritional deficits and responses to nutritional therapy similar to those of the noncancer group, indicating that increases in RBC putrescine during TPN were related to the presence of neoplastic disease and not to changes in host nutritional status.

There are several reasons for increased RBC putrescine levels during TPN in colorectal patients. One explanation is that TPN provides exogenous nutrients that lead to recruitment of Go tumor cells into the growth fraction. The association of urinary putrescine excretion and the thymidine-labelling index of myeloma cells indirectly supports this theory [14]. On the other hand, increased putrescine production may not necessarily reflect increased tumor proliferation. Our unpublished experiments indicate that in rats with solid tumors growing in log-phase TPN increases RBC putrescine levels without increasing tumor weight or ODC activity. This would suggest that TPN may increase ODC substrate levels, resulting in higher putrescine biosynthesis without influencing tumor proliferation. Another explanation is that TPN may preferentially alter the age of RBC in colorectal carcinoma patients and not in noncancer patients. Cooper *et al.* reported that young RBC had higher putrescine levels than old RBC [27]. An increase in the young RBC fraction during TPN could have resulted in increased RBC putrescine levels. Another possibility is that TPN preferentially altered renal excretion of polyamines in colorectal carcinoma patients. This seems unlikely

since plasma creatinine and BUN levels remained in the normal range for both groups during TPN. However, in tumor-bearing animals ODC activity in host tissues is lower than controls [28]. The results of our study have not established a correlation between increased RBC putrescine levels and tumor proliferation during TPN. Further investigations are in progress to determine the variables that affect polyamine biosynthesis during TPN.

Acknowledgements — The authors gratefully acknowledge the technical assistance of Domitilia Patenia, Tai-Hwa Chang, and Sandra Norman and the assistance of Jeanette Godley and Lore Feldman for preparing and reviewing this manuscript.

REFERENCES

1. Brennan MF. Total parenteral nutrition in the cancer patient. *N Engl J Med* 1981, **305**, 375–382.
2. Stragand JJ, Braunschweiger PG, Pollice AA, Schiffer LM. Cell kinetic alterations in murine mammary tumors following fasting and refeeding. *Eur J Cancer* 1979, **15**, 281–286.
3. Buzby GP, Mullen JL, Stein TP, Miller EE, Hobbs CL, Rosato EF. Host-tumor interaction and nutrient supply. *Cancer* 1980, **45**, 2940–2948.
4. Cameron IL. Effect of total parenteral nutrition on tumor-host responses in rats. *Cancer Treatment Reports* 1981, **65** (suppl 5), 93–99.
5. Popp MB, Morrison SD, Brennan MB. Total parenteral nutrition in a methylcholanthrene induced rat sarcoma model. *Cancer Treatment Reports* 1981, **65**, 137–143.
6. Pegg AE, McCann PP. Polyamine metabolism and function. *Am J Physiol* 1982, **243**, C212–C221.
7. Russell DH. Clinical relevance of polyamines. *CRC Crit Rev Clin Lab Sci* 1983, **18**, 261–311.
8. Russell DH. Increased polyamine concentrations in the urine of human cancer patients. *Nature (Lond) New Biol* 1971, **233**, 144–145.
9. Lipton A, Sheehan LM, Kessler GF. Urinary polyamine levels in human cancer. *Cancer* 1975, **35**, 464–468.
10. Waalkes TP, Gehrke CW, Tormey DC, Zumwalt RW, Hueser JN, Kuo KC, Lakings DB, Ahmann DL, Moertel CG. Urinary excretion of polyamines with advanced malignancy. *Cancer Chemother Rep* 1975, **59**, 1103–1116.
11. Nishioka K, Romsdahl MM, Fritsche HA, Johnston DA. Polyamines in sera of patients with solid tumors. *Adv Polyamine Res* 1978, **2**, 265–272.
12. Takami H, Nishioka K. Raised polyamines in erythrocytes from melanoma-bearing mice and patients with solid tumors. *Br J Cancer* 1980, **41**, 751–756.
13. Marton LJ, Edwards MS, Levin VA, Lubich WP, Wilson CB. CSF polyamines: a new and important means of monitoring patients with medulloblastoma. *Cancer* 1981, **47**, 757–760.
14. Russell DH, Durie BGM, Salmon SE. Polyamines as predictors of success and failure in cancer chemotherapy. *Lancet* 1975, **2**, 797–799.
15. Doumas BT, Biggs HG. Determination of serum albumin. *Stand Methods Clin Chem* 1972, **7**, 175–188.
16. Goodgame JT, Lowry SF, Brennan MF. Nutritional manipulations and tumor growth. II. The effects of intravenous feeding. *Am J Clin Nutr* 1979, **32**, 2285–2294.
17. Kishi T, Iwasawa Y, Itoh H, Chibata I. Nutritional responses of tumor-bearing rats to oral or intravenous feeding. *J Parenteral Enteral Nutrition* 1982, **6**, 295–300.
18. Hak LJ, Raasch RH, Hammer VB, Matthews T, Sandler RS, Heizer WD. Comparison of intravenous glucose and fat calories on host and tumor growth. *J Parenteral Enteral Nutrition* 1984, **8**, 657–659.
19. Norton L, Simon R. Tumor size, sensitivity to therapy and design of treatment schedules. *Cancer Treatment Rep* 1977, **61**, 1307–1317.
20. Bachrach U, Don S, Wiener H. Polyamines in normal and in virus-transformed chick embryo fibroblast. *Cancer Res* 1974, **34**, 1577–1580.
21. Don S, Wiener H, Bachrach U. Specific increase in polyamine levels in chick embryo cells transformed by Rous sarcoma cells. *Cancer Res* 1975, **35**, 194–198.
22. Haddox MK, Magun BE, Russell DH. Ornithine decarboxylase induction during G_1 progression of normal and Rous sarcoma virus-transformed cells. *Cancer Res* 1980, **40**, 604–608.
23. Shipe JR, Savory J, Willis RR, Rowley R, Looney WB, Hopkins HA. Erythrocyte polyamine levels in rats with H4IIE hepatomas before and after radiation treatment. *Res Commun Chem Pathol Pharmacol* 1980, **28**, 329–342.
24. Durie BGM, Salmon SE, Russell DH. Polyamines as markers of response and disease activity in cancer chemotherapy. *Cancer Res* 1977, **37**, 214–221.
25 Casti A, Orlandini G, Reali N, Bacciottini F, Vanelli M, Bernasconi S. Pattern of blood polyamines in healthy subjects from infancy to the adult age. *J Endocrinol Invest* 1982, **5**, 263–266.
26. Ota DM, Nishioka K, Foulkes M, Grossie VB. Nutritional parameters affecting erythrocyte polyamine levels in cancer patients. *J Clin Oncology* 1984, **2**, 1157–1164.

27. Cooper KD, Shukla JB, Rennert OM. Polyamine distribution in cellular compartments of blood and in ageing erythrocytes. *Clin Chim Acta* 1976, **73**, 71–88.
28. Grossie VB, Nishioka K, Ota DM, Ajani JA, Chang T-H. Ornithine decarboxylase activity of normal tissue from tumor-bearing rats. *J Nutrition, Growth and Cancer.* In press.