peptides, oligopeptides, small peptides, and free amino acids (*2*). Protein digestion is generally an efficient system in which most of the protein products of digestion found in the portal vein are free amino acids. However, numerous investigators have reported detection, in the systemic circulation, of small amounts of dietary dipeptides, tripeptides, and even minute amounts of protein fragments and intact proteins, all from the intestine. Gelatin has been shown to increase the urinary excretion of the dipeptide prolylhydroxyproline and the tripeptide glycylprolyhydroxyproline of human volunteers (*3*). It has even been reported that isolated loops of adult rat small intestine, which had been injected with insulin, caused hypoglycemia, indicating that it was absorbed in a biologically active form (*4*).

It has been demonstrated that a soy protein isolate, the Bowman Birk protease inhibitor, becomes widely distributed in the tissue of mice following oral gavage. This inhibitor was found in the liver, blood, lung, kidney, and urine by using radioactive techniques (*5*). Immunological techniques have been used to quantitatively measure β-lactoglobulin in the serum of nonallergic healthy university students after a "milk load" (*6*). More extensive reviews are available on this subject (*4,7,8*).

Some of the components of shark cartilage may similarly escape digestion, enter the blood stream, and find their way to a cancer site by the blood circulation system. By this mechanism, an orally ingested protein could exert a biological effect on a tumor outside the gastrointestinal tract. Whether shark cartilage exerts a biological effect on tumors by this route of administration remains to be demonstrated.

Skepticism is certainly warranted on any new cancer therapy, but let us not be too quick to dismiss anyone's work.

C. BRIAN BLACKADAR, M.D.
Department of Nutritional Sciences
University of Guelph
Guelph, ON N1G 2W1
Canada

References

(*1*) Mathews J: Sharks still intrigue cancer researchers [published erratum appears in J Natl Cancer Inst 84:1237, 1992]. J Natl Cancer Inst 84:1000–1002, 1992
(*2*) Hunt SM, Groff JL: Advanced nutrition and metabolism. *In* Proteins. New York: West Publishing Co. 1990, pp 129–164
(*3*) Adibi SA, Kim YS: Peptide absorption and hydrolysis. *In* Physiology of the Gastrointestinal Tract (Jonso LR, ed). New York: Raven Press, 1981, pp 1073–1095
(*4*) Walker WA: Intestinal transport of macromolecules. *In* Physiology of the Gastrointestinal Tract (Johnso LR, ed). New York: Raven Press, 1981, pp 1271–1289
(*5*) Billings PC, St. Clair WH, Maki PA, et al: Distribution of the Bowman Birk protease inhibitor in mice following oral administration. Cancer Lett 62:191–197, 1992
(*6*) Lovegrove JA, Osman DL, Morgan JB, et al: Transfer of cow's milk β-lactoglobulin to human serum after a milk load: a pilot study. Gut 34:203–207, 1993
(*7*) Gardner ML: Gastrointestinal absorption of intact proteins. Annu Rev Nutr 8:329–350, 1988
(*8*) Sanderson IR, Walker WA: Uptake and transport of macromolecules by the intestine: possible role in clinical disorders (an update). Gastroenterology 104:622–639, 1993

Sequential High-Dose Combination Chemotherapy With Granulocyte Colony-Stimulating Factor and Peripheral Blood Progenitor Cells in Patients With Solid Tumors: Intensification Limited by Nonhematologic Toxic Effects

In solid tumors, dose-intensive treatments have often been given late in the course of therapy by using a single cycle of high-intensity chemotherapy with the support of autologous bone marrow transplantation (*1*). Relapses occur, however, in the majority of patients, suggesting the selection of resistant tumor clones. Early delivery of multiple and sequential courses of high-dose combination chemotherapy might produce superior cell kill and decrease the emergence of tumor-cell resistance (*2*). In recent years, the use of hematopoietic growth factors has reduced the duration of myelosuppression induced by chemotherapy (*3*). These agents also expand the pool of circulating peripheral blood progenitor cells that can be harvested by leukapheresis and reinfused following high-dose chemotherapy (*4,5*).

We designed a study in which repeated courses of high-dose combination chemotherapy were administered sequentially with the support of peripheral blood progenitor cells and granulocyte colony-stimulating factor (G-CSF). Our purposes were (*a*) to investigate whether G-CSF alone could mobilize a sufficient number of peripheral blood progenitor cells for multiple treatment cycles and (*b*) to define the limiting toxic effects of such an approach. This study was approved by the ethical committee of our institution, and all the patients gave written informed consent. Mobilization of peripheral blood progenitor cells was performed prior to chemotherapy by G-CSF (filgrastim) alone, given subcutaneously at a dose of 12 μg/kg per day for a median of 8 days (range, 7-10 days). The median number of leukaphereses was four (range, three to five). This design allowed collection of a median number of mononuclear cells equal to 5.5×10^8/kg (range, $3.6\text{-}19.9 \times 10^8$/kg), which is equivalent to 53.2×10^4/kg granulocyte–macrophage colony-forming units (GM-CFU) (range, $41.4\text{-}210.1 \times 10^4$/kg) and 5.2×10^6/kg CD34-positive cells (range, $0.6\text{-}26.5 \times 10^6$/kg).

The chemotherapy program consisted of 2500 mg/m^2 cyclophosphamide on days 1 and 2; 300 mg/m^2 etoposide on days 1, 2, and 3; and 50 mg/m^2 cisplatin on days 1, 2, and 3. This treatment program was to be repeated every 21 days for four cycles. Mesna (4 g/m^2 per day) was given as a continuous intravenous infusion on days 1 and 2. The projected average dose intensity was 4.4 times higher (*6*) than the standard regimen, which is repeated every 21 days for six cycles. Peripheral blood progenitor cells were reinfused 24-48 hours after the end of each chemotherapy cycle, with G-CSF support at 12 μg/kg per day subcutaneously for 14 days or less if early recovery occurred.

Between June 1992 and January 1993, seven patients were enrolled in the study. Five patients had limited

small-cell lung cancer (SCLC), one had extensive SCLC, and one had metastatic breast cancer. We were able to evaluate 21 cycles of therapy. Three patients completed the four cycles that constituted full treatment; two completed three cycles; one completed two cycles; and one completed one cycle. The hematologic toxic effects are summarized in Table 1. Overall median times to recovery of leukocyte recovery counts to at least 0.3, 0.5, or 1.0 × 10^9/L were 9, 10, and 10 days, respectively. This period was calculated from the day of peripheral blood progenitor cell reinfusion. The median time was 6 days with a leukocyte count of 0.3 × 10^9/L or less, 7 days with a leukocyte count of 0.5 × 10^9/L or less, and 8 days with a leukocyte count of 1.0 × 10^9/L or less.

Thrombocytopenia did not last long and did not cause clinically significant bleeding. The median times to recovery of platelet counts to 10 × 10^9/L or more and 20 × 10^9/L or more were 10 and 11 days, respectively; the median times with platelet counts of 10 × 10^9/L or less and 20 × 10^9/L or less were 4 and 6 days, respectively. The durations of neutropenia and thrombocytopenia were not significantly different between the first and the fourth cycles. The median number of GM-CFU/kg reinfused after each cycle of chemotherapy was 6.6 × 10^4/kg (range, 1.7-61.0 × 10^4/kg). There was a direct relationship between the number of reinfused GM-CFU and recovery of bone marrow function. When the number of GM-CFU was 10 × 10^4/kg or less, the median time to reach a leukocyte count of 1.0 × 10^9/L or more was 11 days (range, 10-15 days). It was 9 days (range, 8-10 days) with a GM-CFU count of 10 × 10^4/kg or more ($P<.001$). The median time to platelet counts of 20 × 10^9/L or more was 13 days (range, 9-15 days) after 10 × 10^4/kg or less GM-CFU and 10 days (range, 9-12 days) after more than 10 × 10^4/kg reinfused GM-CFU ($P>.05$).

The limiting toxic effects of this treatment protocol were neurotoxic effects and ototoxic effects after the third and fourth treatment cycles. The three patients who received four cycles of treatment developed World Health Organization (WHO) (*7*) grade 2 ototoxic effects and a median of WHO grade 3 neurotoxic effects (range, grades 2-3). These toxic effects prompted us to cancel the fourth cycle in subsequent patients. The levels of leukocyte and platelet counts would have allowed treatment every 21 days, but a 1-week pause was required in all patients who complained of severe exhaustion. The median weight loss was 3.5 kg (range, 2-8 kg) after one cycle and increased to 8 kg (range, 7-11 kg) after the fourth cycle. Other toxic effects consisted of moderate nausea and vomiting at the time of chemotherapy administration and during the following days. Mucositis and diarrhea were mild except in two patients who developed grade 3 esophagitis and in one patient who had an episode of transient ileus with bleeding. Among the six patients with SCLC, four had complete response and two had partial response, according to WHO criteria (*7*). The patient with metastatic breast cancer obtained an unmaintained complete remission lasting more than 12 months.

This correspondence describes the feasibility of delivering multiple and sequential courses of a combination high-dose chemotherapy regimen with G-CSF and peripheral blood progenitor cell protection. However, the nonhematologic toxic effects became the dose-limiting toxic effects. Similar findings were described when carboplatin alone was given in repeated courses (*8,9*). The dose intensity of our regimen was 62% of the projected schedule, but it was 2.7 times higher than that of a standard regimen. To further increase dose intensity, the use of agents that have mostly hematologic toxic effects might be preferable in the design of high-dose combination protocols, and treatments for the protection of normal tissues

Table 1. Hematologic toxic effects

	Cycle No.							
	1		2		3		4	
Median No. of days (range) to leukocyte recovery*								
≥0.3 × 10^9/L	8	(8-11)	8.5	(7-10)	9	(8-10)	10	(9-11)
≥0.5 × 10^9/L	9	(9-14)	10	(8-11)	11	(8-12)	10	(9-11)
≥1.0 × 10^9/L	10	(9-15)	10	(8-11)	10	(9-12)	10	(10-12)
Median No. of days (range) with leukocyte count								
≤0.3 × 10^9/L	6	(5-9)	5.5	(4-8)	6	(6-7)	7	(6-8)
≤0.5 × 10^9/L	8	(6-12)	6.5	(5-9)	7	(5-9)	7	(6-9)
≤1.0 × 10^9/L	9	(7-13)	7.5	(6-10)	7	(6-9)	10	(8-11)
Median No. of days (range) to platelet recovery*								
≥10 × 10^9/L	10	(7-14)	9	(9-12)	10	(7-11)	12	(9-12)
≥20 × 10^9/L	10	(9-15)	11	(9-12)	12	(10-14)	13	(9-14)
Median No. of days (range) with platelet count								
≤10 × 10^9/L	5	(1-10)	5	(1-5)	4	(1-5)	7	(1-7)
≤20 × 10^9/L	6	(1-11)	4	(3-8)	6.5	(6-9)	7	(2-9)
Median No. (range) of platelet transfusions	3.5	(1-9)	1.5	(1-3)	2	(2-3)	3	(1-4)
Median No. (range) of red blood cell transfusions	1	(0-4)	1.5	(0-2)	2	(1-3)	2	(2-4)
Median No. of days (range) of hospitalization	18	(15-44)	18	(16-23)	19	(15-20)	18	(18-20)
Median No. of days (range) with intravenous antibiotics	11	(6-14)	8	(0-14)	8	(0-14)	6	(0-8)

*Number of days calculated from day of reinfusion of peripheral blood progenitor cells.

should also be developed (*10*).

SERGE LEYVRAZ
NICOLAS KETTERER
PATRICIA VUICHARD
VLADIMIR VON FLIEDNER
FERDY LEJEUNE
Centre Pluridisciplinaire d'Oncologie
PHILIPPE SCHNEIDER
Transfusion Center,
Croix-Rouge Suisse
JEAN-PHILIPPE GROB
FEDOR BACHMANN
Hematology Department
University Hospital
Lausanne, Switzerland

References

(*1*) Antman KH, Souhami RL: High-dose chemotherapy in solid tumours. A review of published data in selected tumours with a commentary. Ann Oncol 4 (Suppl 1):29–44, 1993

(*2*) Ragaz J, Goldie J, Coldman A, et al: Kinetic and biological effects of high-dose intensity consolidation chemotherapy regimen for solid tumor requiring autologous bone marrow, peripheral stem cell support. Rationale for multiple transplants. Proc ASCO: 301, 1991

(*3*) Crawford J, Ozer H, Stoller R, et al: Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small-cell lung cancer. N Engl J Med 325:164–170, 1991

(*4*) Socinski MA, Cannistra SA, Elias A, et al: Granulocyte-macrophage colony stimulating factor expands the circulating haemopoietic progenitor cell compartment in man. Lancet 1:1194–1198, 1988

(*5*) Sheridan WP, Begley CG, Juttner CA, et al: Effect of peripheral-blood progenitor cells mobilised by filgrastim (G-CSF) on platelet recovery after high-dose chemotherapy. Lancet 339:640–644, 1992

(*6*) Cortes Funes H, Dominguez P, Torrubia AP, et al: Treatment of small cell lung cancer with a combination of VP16-213 and cyclophosphamide with cisplatin or radiotherapy. Cancer Chemother Pharmacol 7:181–186, 1982

(*7*) World Health Organization: WHO Handbook for Reporting Results of Cancer Treatment. Offset Publ No. 48. Geneva: WHO, 1979

(*8*) Shea TC, Mason JR, Stomiolo AM, et al: Sequential cycles of high-dose carboplatin administered with recombinant human granulocyte-macrophage colony-stimulating factor and repeated infusions of autologous peripheral-blood progenitor cells: a novel and effective method for delivering multiple courses of dose-intensive therapy. J Clin Oncol 10:464–473, 1992

(*9*) Crown J, Wassherheit C, Hakes T, et al: Rapid delivery of multiple high-dose chemotheraphy courses with granulocyte colony-stimulating factor and peripheral blood-derived hematopoietic progenitor cells. J Natl Cancer Inst 84:1935–1936, 1992

(*10*) van der Hoop RG, Vecht CJ, van der Burg ME, et al: Prevention of cisplatin neurotoxicity with an ACTH(4-9) analogue in patients with ovarian cancer. N Engl J Med 322:89–94, 1990

Notes

Correspondence to: Serge Leyvraz, M.D., Centre Pluridisciplinaire d'Oncologie, CHUV — BH 10, rue du Bugnon 46, 1011 Lausanne, Switzerland.

We thank Prof. M. Glauser and Dr. M. Cometta from the Infectious Disease Department for help in caring for these patients, and Ms. M. Gonin for secretarial assistance.

Selenium and DDE in Breast Fat of Breast Cancer Patients: Their Relationship to Hormone Receptors in Breast Tissue

In recent studies, higher concentrations of neutral organochlorine DDE (1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene) and PCB (polychlorinated biphenyls) have been found in breast fat or serum of breast cancer patients than in control subjects (*1-3*). We have also investigated the occurrence of certain neutral organochlorine compounds and antioxidants (selenium [Se], copper [Cu], and zinc [Zn]). Only residues of β-hexachlorocyclohexane were significantly more frequent in cancer patients than in controls (P = .03) (*4*). When parity and age were adjusted for, the odds ratio was 10.51 (95% confidence interval [CI] = 2.00-55.26) for patients whose breast adipose tissue had β-hexachlorocyclohexane of more than 0.1 mg/kg fat.

In rat and mouse studies, Se has had an inhibiting effect on carcinogen-induced and spontaneous mammary gland cancer (*5-7*). Inhibition of mammary tumorigenesis requires the continuous presence of Se. After withdrawal of the Se supplement, mammary tumorigenesis reappears at the same rate as tumors in the control mice and rats (*8*). In breast cancer patients, however, the finding of lower levels of Se in serum has not been consistent (*9*).

We (*10*) found that the mean Se concentration in the breast tissue of cancer patients was nearly statistically significantly higher than in the breast tissue of healthy controls (P = .06). This finding is in accordance with a mouse study by Lane and Medina (*7*) suggesting that breast cancer tissue may concentrate Se.

Here, we report our results on the relationship of Se, Cu, and Zn and certain organochlorine compounds to the amount of estrogen receptors (ERs) and progesterone receptors (PRs) found in the cancer tissue (Table 1). The study material consisted of adipose samples of breast tissue of 44 breast cancer patients aged 34-82 years (mean, 56 years; SD ± 11 years) who were residents of the Helsinki area [reported in detail (*4,10*)]. Of these carcinomas, 76% were ductal. Stage of the disease was recorded using the UICC system of staging (*11*). Organochlorine concentrations were detected by mass spectrometry and concentrations of antioxidants by atomic absorption. The ER and PR levels were measured in 30 patients by the method reported by Vihko et al. (*12*).

A positive correlation was found between Se (μg/kg breast tissue) and ER (fmol/mg cytosol protein) (r_s = .480; 95% CI = .14-.72; P = .007). When patients whose disease was classified as stage IV (i.e., distant metastases) were omitted (three cases), a positive correlation was found between DDE and ER concentrations (r_s = .490; 95% CI = .14-.74; P = .009). β-Hexachlorocyclohexane had no association with ER, in accordance with in vitro studies by Coosen and van Velsen (*13*).

Our results suggest that Se may act via ER. The positive association of DDE with ER may indicate that the carcinogenic effect of DDE is indirect and may also be associated with ER. This connection between ER and pollutants like DDE may also explain the recent increase in ER-positive breast cancers compared with ER-negative breast cancers (*14*).

H. MUSSALO-RAUHAMAA
Department of Public Health
University of Helsinki