

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 glycyprolylhydroxyproline 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.

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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 μ g/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 - 19.9×10^8 /kg), which is equivalent to 53.2×10^4 /kg granulocyte-macrophage colony-forming units (GM-CFU) (range, 41.4 - 210.1×10^4 /kg) and 5.2×10^6 /kg CD34-positive cells (range, 0.6 - 26.5×10^6 /kg).

The chemotherapy program consisted of 2500 mg/m² cyclophosphamide on days 1 and 2; 300 mg/m² etoposide on days 1, 2, and 3; and 50 mg/m² cisplatin on days 1, 2, and 3. This treatment program was to be repeated every 21 days for four cycles. Mesna (4 g/m² 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 μ g/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 $0.3 \times 10^9/L$, $0.5 \times 10^9/L$, or $1.0 \times 10^9/L$ 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 $0.3 \times 10^9/L$ or less, 7 days with a leukocyte count of $0.5 \times 10^9/L$ or less, and 8 days with a leukocyte count of $1.0 \times 10^9/L$ or less.

Thrombocytopenia did not last long and did not cause clinically significant bleeding. The median times to recovery of platelet counts to $10 \times 10^9/L$ or more and $20 \times 10^9/L$ or more were 10 and 11 days, respectively; the median times with platelet counts of $10 \times 10^9/L$ or less and $20 \times 10^9/L$ 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 chemo-

therapy was $6.6 \times 10^4/kg$ (range, $1.7-61.0 \times 10^4/kg$). 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 $10 \times 10^4/kg$ or less, the median time to reach a leukocyte count of $1.0 \times 10^9/L$ or more was 11 days (range, 10-15 days). It was 9 days (range, 8-10 days) with a GM-CFU count of $10 \times 10^4/kg$ or more ($P < .001$). The median time to platelet counts of $20 \times 10^9/L$ or more was 13 days (range, 9-15 days) after $10 \times 10^4/kg$ or less GM-CFU and 10 days (range, 9-12 days) after more than $10 \times 10^4/kg$ 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 non-hematologic 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*				
$\geq 0.3 \times 10^9/L$	8 (8-11)	8.5 (7-10)	9 (8-10)	10 (9-11)
$\geq 0.5 \times 10^9/L$	9 (9-14)	10 (8-11)	11 (8-12)	10 (9-11)
$\geq 1.0 \times 10^9/L$	10 (9-15)	10 (8-11)	10 (9-12)	10 (10-12)
Median No. of days (range) with leukocyte count				
$\leq 0.3 \times 10^9/L$	6 (5-9)	5.5 (4-8)	6 (6-7)	7 (6-8)
$\leq 0.5 \times 10^9/L$	8 (6-12)	6.5 (5-9)	7 (5-9)	7 (6-9)
$\leq 1.0 \times 10^9/L$	9 (7-13)	7.5 (6-10)	7 (6-9)	10 (8-11)
Median No. of days (range) to platelet recovery*				
$\geq 10 \times 10^9/L$	10 (7-14)	9 (9-12)	10 (7-11)	12 (9-12)
$\geq 20 \times 10^9/L$	10 (9-15)	11 (9-12)	12 (10-14)	13 (9-14)
Median No. of days (range) with platelet count				
$\leq 10 \times 10^9/L$	5 (1-10)	5 (1-5)	4 (1-5)	7 (1-7)
$\leq 20 \times 10^9/L$	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).

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Notes

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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; $SD \pm 11$ 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 ($\mu\text{g/kg}$ 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).

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