Effects of Administration of Oral Branched-Chain Amino Acids on Anorexia and Caloric Intake in Cancer Patients

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Anorexia, with its attendant reduction of food intake and progressive depletion of body stores, is among the major causes of the anorexia-cachexia syndrome occurring in cancer patients (1). Results from a number of studies (2-6) suggest that the enhanced brain availability of the amino acid precursor of serotonin, tryptophan, may play a role in the pathogenesis of cancer anorexia by increasing brain serotoninergic activity.

During tumor growth, plasma-free tryptophan concentrations, one of the major determinants of brain tryptophan concentrations and serotonin synthesis, are significantly increased (2-4). Tryptophan entry into the brain is regulated by a specific transport system, which is competitively shared by the other large neutral amino acids (i.e., branched-chain amino acids [BCAAs], tyrosine, phenylalanine, and methionine). Thus, it is conceivable that entry of tryptophan into the brain may be reduced by increasing the plasma levels of competitors of tryptophan (7). We therefore hypothesized that the oral administration of BCAAs to cancer patients with anorexia would lead to decreased brain tryptophan concentrations and reduced serotoninergic activity, eventually resulting in an improvement of food intake.

To test this hypothesis, 28 anorexic, not weight-losing patients, who had been admitted to our institutions in Italy with newly diagnosed, resectable cancers and who were undergoing surgical resection of the tumors, were enrolled in the study. None of the patients received radiotherapy and/or chemotherapy during the study or in the previous 4 weeks.

The research protocol, double-blinded and placebo-controlled, was approved by the ethics committees at our institutions in Italy. After giving written, informed consent prior to surgery, patients were randomly assigned to receive either 4.8 g three times per day of a BCAA mixture (leucine, 2.36 g; isoleucine 1.28 g, and valine 1.16 g; BCAA group; n = 15 patients) or a placebo (isotensive dose of glycine; placebo group; n = 13 patients), both obtained from Bracco Industria Chimica, Milan, Italy. BCAAs and a placebo, in powder form, were dissolved in tap water, and patients were required to take them orally three times per day, 60 minutes before each meal, for 7 consecutive days.

Nutritional status prior to and at the end of the study was investigated by means of biochemical indices (serum levels of C3, prealbumin, transferrin, and ceruloplasmin). Daily caloric intake was evaluated in each patient throughout the study by carefully weighing food before and after each meal. The presence of anorexia was investigated prior to and at the end of the study, using a previously described questionnaire (4).

In the placebo group, plasma amino acid patterns did not change throughout the study period (Fig. 1, A and B). In the BCAA group, plasma large neutral amino acids (LNAA) increased significantly (Fig. 1, A), mainly as a consequence of an increase in BCAA concentrations (104.7 ± 14.4 μmol/dL on day +7; +121% versus base line; P<.01). Consequently, the free tryptophan/LNAA ratio, which closely predicts brain tryptophan concentrations (5), decreased significantly in patients receiving BCAA (Fig. 1, B).

The incidence of anorexia decreased significantly among patients in the BCAA group when compared with the base line (100% prior to and 45% at the end of the study; P<.05), while it did not change among patients in the placebo group (100% versus 84%, respectively). When compared with the base line, caloric intake by the patients increased significantly in the BCAA group but remained unchanged in the placebo group (Fig. 2).

Cancer anorexia impinges significantly on quality of life, reduces the benefits of antineoplastic therapy, and ultimately for reasons not related to the trial. Assessable patients in the placebo (n = 12) and BCAA (n = 13) groups were comparable for sex (male to female ratio = 7:5 and 7:6, respectively), age (64.6 ± 3.1 years [range, 41-81 years] and 65.0 ± 3.1 years [range, 47-83 years], respectively), and tumor origin (lung/gastrointestinal tract/urinary bladder/pancreas/ breast/neck; 4/4/1/1/1 and 5/5/1/1/0, respectively). In all patients, the biochemical indices of nutritional status were within the normal range prior to and at the end of the study (data not shown).

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influences the patient's overall outcome (8, 9). Thus, a number of drugs have been tested in the treatment of the anorexia-cachexia syndrome, with conflicting results (10).

Previous results from our laboratory (11) implicated increased brain tryptophan availability as a major determinant of anorexia occurring in several diseases. The present data are in agreement with this reasoning and indicate that BCAAs may be used safely to improve caloric intake in cancer patients with anorexia. This is probably the consequence of a reduction of tryptophan brain entry and serotonin synthesis.

Because of the small sample size, the results of this study should be considered as preliminary and should be confirmed by further clinical trials involving a larger series of patients. However, considering the paucity of therapeutic resources for cancer anorexia, this new approach seems very encouraging.

References

Inherited mutations in the BRCA1 breast-ovarian cancer gene, isolated in 1994 (1), have been estimated to be responsible for approximately 5% of ovarian cancer diagnosed in women under the age of 50 years (2). Recently, the BRCA2 gene was identified (3), and it appears to confer a much lower risk of ovarian cancers (2,4). BRCA1 and BRCA2 are believed to be tumor suppressor genes, since both alleles appear to be inactivated during neoplastic development (5,6). Despite extensive BRCA1-screening efforts, only five somatic and 11 germline mutations have been identified among 267 sporadic (i.e., nonfamilial) ovarian cancers studied to date (7-11). As BRCA1 is a large gene with widely distributed alterations, mutation screening is labor intensive. Approximately 90% of the reported BRCA1 alterations are either frameshift or nonsense mutations, which result in the premature termination of protein synthesis (12-15). Therefore, we developed the protein truncation test as an efficient strategy to determine the role of BRCA1 mutations in patients with early-onset ovarian cancer.

Epithelial ovarian tumor tissue and matched blood lymphocytes were obtained, with institutional review board-approved, written informed consent, from 16 patients treated at the Division of Gynecologic Oncology at Duke University Medical Center. The mean age of the patients at disease onset was 48 years. Exon 11 (61% of coding sequence) was screened by the protein truncation test using primers and protocols that are available on-line in the Breast Cancer Information Core database (http://www.ncbi.nlm.nih.gov/ntmuseum_research/lab_transfer/BIC/) (16). In addition, the entire BRCA1-coding region of these tumor DNAs was analyzed by single-strand conformation analysis, as previously described (7,16).

Analysis using the protein truncation test revealed alterations in BRCA1 protein size in patients EOO473 and EO01906 (Fig. 1, A). Direct DNA sequence-sequencing identified an identical, previously unreported, single base-pair deletion, 2575delC, in exon 11, causing a frameshift and subsequent premature stop codon (Table 1). This deletion was present in the germline of both patients (data not shown) who were apparently unrelated. Patient EOO473 had no known family history of cancer, but patient EO01906 reported a strong family history (Table 1). In patient EO023, single-strand conformation analysis of tumor DNA revealed a mobility shift that was shown by DNA sequencing to result from a T→G transversion 11 base pairs prior to the 3' splice site of intron 5 (Table 1). This previously described alteration (14,15) activates a cryptic splice site that includes 59 nucleotides of intron 5 in the BRCA1 messenger RNA (mRNA) of this patient. This mutation was also detectable by the protein truncation test using tumor complementary DNA (Fig. 1, B). Direct sequencing of lymphocyte DNA indicated that this was a germline alteration (data not shown), and this patient reported a sister with dual primary breast and ovarian cancers (Table 1).

In this study, we have identified germline BRCA1 mutations in three of 16 women with early-onset ovarian cancer. Despite extensive searches by several groups (7-11), somatic mutations in BRCA1 appear to be quite rare. It is interesting that the sporadic ovarian cancer patients with germline mutations described previously (10,11) and in this brief communication (average age at disease onset = 45 years) developed cancer approximately 18 years earlier than patients with somatic mutations (average age at disease onset = 63 years) (8,9). This significant age difference at disease diagnosis (Student's t test, P<0.003) is consistent with the need for two somatic events to inactivate BRCA1.

Although mutation detection in BRCA1 remains technically challenging, the results of the current study and those obtained by other groups (17,18) suggest that the protein truncation test is an efficient screening tool. This test does have certain shortcomings, however. In this test, missense alterations are undetectable, and sensitivity is limited for mutations that yield either short or very large products. Adapting gel concentrations and electrophoresis times as well as substituting [3H]leucine for [35S]methionine may help to circumvent these problems (17,19). Screening the entire coding region of the gene by the protein truncation test requires the availability of cellular RNA, whereas only exon 11 can be screened using genomic DNA. Finally, in specific cases, the instability of mRNA-containing, premature stop codons may also reduce detection by this technique (20).

In summary, although somatic BRCA1 mutations are responsible for only a