NOTE
DNA-Synthesis-Stimulating Activity in
the Sera of Anorexia Nervosa

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Abstract

Serum factors which stimulate DNA synthesis were investigated in patients with anorexia nervosa. The factors were assayed by examining [^3H]-thymidine incorporation into the nuclei of human fibroblasts in culture using autoradiography. The mean percentage of labeled nuclei (labeling index) induced by serum was significantly lower ($p < 0.005$) in 5 patients with anorexia nervosa (21.0 ± 5.9 percent, mean $\pm$ S.D.) than in 5 normal adult subjects (33.8 ± 3.3 percent). Two of the patients with anorexia nervosa were treated with intravenous hyperalimentation (IVH). In one of the IVH treated patients, the labeling index increased from 17.4 to 32.8 percent at the 6th week of treatment, and in the other increased from 19.4 to 29.8 percent at the 4th week. In both patients, body weight increased by 1.5 to 2 kg. IVH in these patients increased DNA-synthesis-stimulating activity.

The effect of numerous growth factors on DNA synthesis in cultured human fibroblasts have been studied. These include studies on insulin-like growth factor I and II, somatomedin A, rat multiplication-stimulating activity, epidermal growth factor and fibroblasts growth factor as well as insulin (Rechler *et al.*, 1974, Rechler *et al.*, 1978, Rose *et al.*, 1975, Gospodarowicz, 1974). In an early study of the relation between nutrition and growth factor, Daughaday and Kipnis (1966) reported that sulfation factor activity in sera decreased following a 48 hour fast in rat. Phillips and Young (1976) found that the somatomedin activity level decreased in rats not only during fasting but also in association with malnutrition without complete deprivation of food. In humans, somatomedin (sulfation factor) activity is depressed in malnourished

children (Hintz *et al.*, 1978) and in patients with anorexia nervosa (Rappaport *et al.*, 1978). According to the reports of Van Wyk *et al.* (1972), the plasma factors which stimulate sulfate incorporation into cartilage are the same as those that are responsible for the synthesis of DNA. It has not been documented, however, that the serum growth factors which stimulate DNA synthesis in human fibroblasts are low in the sera obtained from malnourished humans. We applied the method of autoradiography to evaluate DNA-synthesis-stimulating activity by measuring thymidine incorporation into the nuclei of fibroblasts in culture. This paper describes the level of DNA-synthesis-stimulating activity in sera obtained from patients with anorexia nervosa and its change following IVH.

Table 1. Clinical features and laboratory findings in patients with anorexia nervosa

Patient No.	1	2	3	4	5
Age (yr)	20	21	17	14	20
Sex	F	F	F	F	F
Height (cm)	162	152	144	159	158
Weight (kg)	30	34	29	35	38
% ideal weight	-46%	-27%	-27%	-34%	-27%
Total Protein (g/dl)	3.9	7.4	6.8	7.0	5.2
A/G ratio	1.4	1.4	1.7	1.4	1.5
FBS (mg/dl)	74	79	60	84	60
GH (ng/ml)	60.0	6.0	3.8	6.2	80.0
IRI (μ U/ml)	<1.0	7.0	5.0	—	1.0
LH mIU/ml)	1.0	3.8	4.0	3.0	3.6
FSH (mIU/ml)	1.5	7.4	1.8	1.0	2.0
TSH (μ U/ml)	3.6	1.0	2.4	2.4	6.9
T ₃ (ng/ml)	43	65	38	151	42
T ₄ (μ g/ml)	4.2	5.7	5.7	7.0	5.9
Cortisol (μ g/dl)	18.5	22.5	25.5	10.0	32.2

Materials and Methods

Subjects

Five female patients, 14 to 21 years old, with anorexia nervosa were studied. Their body weight ranged from 29 to 38 kg (-29 to -46% of ideal weight calculated by a modification of Broca's formula.) The patients' clinical features and laboratory findings are summarized in Table 1. Blood samples were obtained from patients in the morning after an overnight fast. Four healthy men and 1 healthy woman, 25 to 30 years old, were also studied.

Fibroblasts

The biopsies, 2×2×2 mm in size, were put in a 60 mm plastic dish (Falcon) and incubated in Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum (FCS) (Flow Lab.). Fibroblasts were grown in a high humidity atmosphere of 95% air and 5% CO₂ at 37°C until the cells were confluent. They were then split 1:3 or 1:4. Fibroblast cells between the 4th and 10th passages were used for assays.

Assay procedure

(i) *Autoradiography*: Fibroblasts (1×10⁵ cells) were plated in 35 mm diameter plastic dishes and were cultured in MEM containing 10% FCS. After 24 hr, the medium was replaced with serum free MEM for 48 hr. The medium was then removed and the cultures were washed with phosphate buffered saline (PBS). Two ml of MEM, containing the serum sample at a concentration of 5% and 1 μ Ci/ml of [³H]-thymidine, was added to the cultures. After 24 hr, the cells attached to the dishes were

washed in PBS and fixed in metanol. Acid-soluble materials were removed by washing the cells with 5% trichloroacetic acid for 1 hr at 4°C. The fixed cells were washed with water for 30 min. Then the preparations were dipped in NBT-3 nuclear track emulsion (Eastman Kodack Co.) and exposed for 4 days at 4°C. Nuclei that incorporated [³H]-thymidine into DNA were densely labeled (Fig. 1). The preparations were then stained with Giemsa (MERCK). Cells in which [³H]-thymidine was incorporated into nuclei were called 'positive nuclei'. The labeling index in our study was defined as the percentage of positive nuclei in 1200 cells.

(ii) [³H]-thymidine incorporation: Fibroblasts (1×10⁵ cells) were inoculated in 35 mm diameter plastic dishes and were cultured in MEM containing 10% FCS. After 24 hr, the medium was replaced with serum free MEM for 48 hr. The medium was then removed and MEM containing test serum samples was added to the cultures. After the cultures were exposed to the control serum or sample serum for a period of 18 hr, the cells were pulsed, for 1 hr, with [³H]-thymidine (1 μ Ci/ml in 1 ml media). The radioactive media were removed and the cultures were washed first with PBS two times, then with 3 ml cold 5% trichloroacetic acid and finally with 3 ml of ethanol: ether (3:1). The dried and fixed cells were dissolved in 0.3 N NaOH, and counted for radioactivity following the method of Moses *et al.* (1978).

Sampling

Blood samples were obtained from veins in the ante cubital fossa of fasting subjects in the morning. Sterile sera were stored at -40°C for later analysis of DNA-synthesis-stimulating activity. All samples were measured for DNA-synthesis-stimulating activity in the same assay. For IVH a silicon rubber catheter

(Toyo Hospital Supply Co.,) was inserted into the infraclavicular subclavian vein and threaded into the superior vena cava. The catheter was fixed to the skin with a single suture, and a dressing applied. The occlusive dressing was replaced twice a week after sterilizing the surrounding skin. The nutrient solution was infused continuously. The basic solution contained 20% glucose, 4% amino acids, electrolytes, minerals and vitamins, but no lipid emulsion. The total volume was 40–50 cc/kg/day. Calory intake per day was started at approximately a dose of 40 Cal/kg and then gradually increased to 60 Cal/kg. Oral intake of liquid food was allowed to each patient at a dose of 400–600 Cal/day at the 3rd week of the treatment with IVH. (Okada *et al.*, 1975, Okada *et al.*, 1976, Wilmore and Dudrick, 1968).

Results

1) Assay evaluation

The index was determined by examining five 100× microscopic fields in each dish. The interassay reproducibility was $100 \pm 23.3\%$ (S.D.). Fig. 2 shows that the labeling index responded when the dose of serum added to the incubation medium was increased from 2 to 20%. When serum was not added, the labeling index was less than 1 percent. The mean labeling index of acromegalic patients was $39.2 \pm 2.5\%$ (mean $\pm$ S.D., $n=5$), that of GH-deficient children was $23.4 \pm 4.9\%$ ($n=8$) and that of GH-

Fig. 2 Effect of serum concentration on labeling index. Labeling time was 24 hrs. ●—● anorexia nervosa, ■—■ normal.

Table 2. Labeling index in normal controls, acromegaly, GH-deficient children with and without treatment of HGH

Subjects	n	Labeling index % (mean $\pm$ S.D.)
Normal controls	5	33.8 ± 3.6
Acromegaly	5	$39.2 \pm 2.5^*$
GH-deficient children	8	$23.4 \pm 4.9^{**}$
GH-deficient children treated with HGH	5	33.4 ± 2.9

* $p < 0.05$ vs Normal controls.

** $p < 0.01$ vs Normal controls.

deficient children treated with HGH was $33.4 \pm 2.9\%$ ($n=5$) (Table 2). These samples were also assayed for the biological activity incorporating [3 H]-thymidine into fibroblasts ([3 H]-thymidine incorporation). As shown in Fig. 3, a good correlation ($r=0.67$, $p < 0.01$) was obtained between the values of labeling index and the radioactivity of [3 H]-thymidine incorporated into fibroblasts.

Fig. 3 Correlation between labeling index and [^3H]-thymidine incorporation in acromegaly (■), GH-deficient children treated with HGH (○) and anorexia nervosa (●).

2) Labeling index in patients with anorexia nervosa

The labeling index of the sera obtained from 5 patients with anorexia nervosa ranged from 14.2 to 28.6% (21.4 ± 6.7), while that from 5 normal subjects ranged from 29.2 to 37.2% (33.8 ± 3.6) (Fig. 4). In patients with anorexia nervosa there was no correlation between the labeling index and serum protein levels.

3) Effect of IVH on the labeling index

In two patients with anorexia nervosa, the serial changes in labeling index were studied while the patients were treated with IVH. In one patient, treated with IVH for a period of 6 weeks, IVH was given initially at a rate of 1,200 Cal/day and

Fig. 4 Comparison of labeling index. Mean labeling index was significantly lower ($p < 0.005$) in patients with anorexia nervosa than in control subjects.

gradually increased to 1,900 Cal/day. Her body weight increased from 30.0 to 31.5 kg after 6 weeks of treatment. The labeling index was initially 17.4% and increased to 18.9, 25.8, 32.8% at the 2nd, 4th and 6th weeks, respectively. The other patient was treated with IVH for a period of 4 weeks. Initially IVH was given at a rate of 1,200 Cal/day and gradually increased to 2,000 Cal/day. Her body weight increased from 34.0 to 36.0 kg at the 4th week of treatment. The labeling index was initially 19.4 percent

Fig. 5 Serial change of labeling index in two patients with anorexia nervosa who were treated with intravenous hyperalimentation. The shadow indicates the normal range of labeling index. ●—● labeling index, ■.....■ body weight.

and increased to 27.6, 28.4 and 34.0% in the 2nd, 3rd and 4th weeks, respectively (Fig. 5).

Discussion

Attention has recently been focused on the relation between nutrition and the activity of growth factors in serum. According

to the experimental results of Tanano *et al.* (1978), nutrition plays an important role in the generation of somatomedin A in rats. Hintz *et al.* (1978) reported that somatomedin A activity is decreased in malnourished children. We found that the sera obtained from patients with anorexia nervosa stimulated DNA synthesis in cultured human fibroblasts less than sera from normal subjects. There are many growth factors in serum which stimulate DNA synthesis in cultured human fibroblasts. Presumably the low labeling index of serum from these patients is attributable to the low activity of serum growth factors which stimulate DNA synthesis. Moses *et al.* (1978) found that human fibroblasts were not suitable cells to measure the activity of somatomedin in serum. However, our data indicate that the DNA-synthesis-stimulating activities of serum acromegalic patients are significantly higher than those of control subjects and those of GH-deficient children are significantly lower. Also there was no significant difference between the labeling index of sera from control subjects and from GH-deficient children treated with HGH. These results suggest that GH-dependent factors may be responsible, at least partly, for DNA-synthesis-stimulating activity in human serum. The possible presence of inhibitors of somatomedin in the sera of malnourished children has been discussed by Hintz *et al.* (1978). We have no data on this point. Phillips and Kipnis (1977) state that somatomedin activity is correlated more closely with insulin than with growth hormone levels. We observed no relationship between basal insulin levels and DNA-synthesis-stimulating activity in a limited number of patients. In conclusion, our result indicate that serum DNA-synthesis-stimulating activity is affected by the nutritional state of the subject. Further investigation will be needed to clarify how nutrition alters the production or release of growth factors into blood.

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