Increase of Protein Synthesis by Uridine Supplement in Lectin-Stimulated Peripheral Blood Lymphocytes and EB Virus-Transformed B Cell Line of Hereditary Orotic Aciduria Type I

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YAZAKI, M., OKAJIMA, K., SUCHI, M., MORISHITA, H. and WADA, Y. *Increase of Protein Synthesis by Uridine Supplement in Lectin-Stimulated Peripheral Blood Lymphocytes and EB Virus-Transformed B Cell Line of Hereditary Orotic Aciduria Type I.* Tohoku J. exp. Med., 1987, **153** (3), 189-195 — A 2 month-old Japanese girl with hereditary orotic aciduria type I was treated with oral uridine supplement. The activities of orotate phosphoribosyltransferase (OPRT) and orotidine-5'-phosphate decarboxylase (ODC) in erythrocytes were 2.7 and 0.4%, respectively, of those in the controls. Megaloblastic anemia, excessive urinary excretion of orotic acid, lymphopenia and decreased number of OKT3 positive lymphocytes on admission were corrected after the uridine supplement. Peripheral blood lymphocytes (PBL) were cultured for 24 hr in RPMI 1640 medium with 10% heat-inactivated fetal calf serum and further stimulated with PHA-P, ConA or PWM in the presence of 10 to 1000 μ M uridine. EB virus-transformed B cell line (LCL) maintained with an optimal concentration of uridine was cultured for 48 hr in uridine free medium and cultured for an additional 48 hr with 1 to 1000 μ M uridine. The incorporations of leucine in to PHA-, ConA- and PWM-stimulated PBL and into LCL of the patient increased in the presence of uridine over 10 μ M, although they did not increase in controls. These data suggest that low protein synthesis might correlate with an immune deficiency in hereditary orotic aciduria type I. ——— uridine supplement; hereditary orotic aciduria type I; immune deficiency

Hereditary orotic aciduria is a rare inborn error of pyrimidine metabolism associated with megaloblastic anemia, retarded growth and excessive urinary excretion of orotic acid. The enzyme defect involves either two sequential enzymes of de novo pyrimidine biosynthesis, orotate phosphoribosyltransferase (OPRT) and orotidine-5'-phosphate decarboxylase (ODC) in type I form of the disease (Huguley et al. 1959; Becroft and Phillips 1965; Rogers et al. 1968; Becroft et al. 1969; Tubergen et al. 1969; Beardmore et al. 1972; Webster et al.

1979; Girot et al. 1983; Morishita et al. 1986), or only ODC in type II form (Fox et al. 1969, 1973).

Oral uridine supplement usually improves clinical and hematological abnormalities. Correction of abnormal RNA and DNA synthesis by uridine supplement has been postulated, though not demonstrated. Three of twelve patients with this disease died from varicella or meningitis (Huguley et al. 1959; Fox et al. 1969; Girot et al. 1983). An immune deficiency has been suspected, although there are conflicting reports (Girot et al. 1983, 1984; Becroft et al. 1984).

In this report, we describe uridine supplement has normalized the number of OKT3 positive lymphocytes and has increased protein synthesis in lectin-stimulated peripheral blood lymphocytes (PBL) and EB virus-transformed B cell line (LCL) in vivo.

CASE REPORT

A 2 month-old Japanese girl with severe anemia and ventricular septal defect was admitted to the Nagoya City University Hospital. Her parents were not related. Blood examination revealed erythrocytes of $1,910,000/\text{mm}^3$, hemoglobin 5.2 g/100 ml, and hematocrit 15.7%. Bone marrow examination revealed megaloblastic change with normal serum vitamin B₁₂ and folic acid. Urinary excretion of orotic acid was 357 mg per day. The activities of OPRT and ODC in erythrocytes were 2.7 and 0.4%, respectively, of those in the controls. The activities of OPRT and ODC in LCL were 7.5 and 0.01%, respectively, of those in the controls. Family study revealed parents were heterozygotes.

METHODS

PBL were isolated by Ficoll-Isopaque gradient centrifugation. LCL was established using B95-8 derived EB virus. PBL were cultured for 24 hr in RPMI 1640 medium (GIBCO, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS) before the assay. 1.25×10^5 PBL were further stimulated with phytohemagglutinin-P (PHA-P) (Difco Laboratories, Detroit, MI, USA; $15 \mu\text{g/ml}$) for 4 days, concanavalin A (ConA) (Miles Laboratories, Inc., Elkhart, IN, USA; $12.5 \mu\text{g/ml}$) for 5 days or pokeweed mitogen (PWM) (GIBCO; diluted 1 to 2400) for 5 days in the presence of $10 \mu\text{M}$ to $100 \mu\text{M}$ uridine in flat-bottomed microtiter well containing $200 \mu\text{l}$ RPMI 1640 with 10% heat-inactivated FCS. LCL maintained with an optimal concentration of uridine was cultured for 48 hr with uridine free medium, and 1×10^5 LCL were cultured for an additional 48 hr with 1 to $1000 \mu\text{M}$ uridine in flat-bottomed microtiter well containing $200 \mu\text{l}$ RPMI 1640 with 10% heat-inactivated FCS. $0.5 \mu\text{Ci}$ of [methyl- ^3H] thymidine (5 Ci/mmole , Amersham Japan, Tokyo) or $0.5 \mu\text{Ci}$ of L-[4, 5- ^3H] leucine (1 Ci/mmole , Amersham Japan) was added to each well 18 hr before the end of the culture. The cultured cells were harvested onto glass fiber filters with a multiple sample harvester. The radioactivity in the cells was counted by a liquid scintillation counter. The concentration of uridine in plasma was determined by high-performance liquid chromatography (Mizuguchi 1983).

RESULTS

Immunological investigations were performed at 3 month-old before oral uridine supplement (Table 1). Levels of serum immunoglobulin were normal for her age. Total lymphocytes were $1,760/\text{mm}^3$ with low number of OKT3 positive

TABLE 1. *Immunological studies*

	Date				Normal value
	7/11/85	12/21/85	6/14/86	10/18/86	
Age at study	3m	8m	1y2m	1y6m	
Uridine intake (mg/kg/day)	No	150	150	300	
Plasma uridine (μ M)	NT	NT	17	30	$2.33 \pm 1.05^*$ ($M \pm s.d.$, $n = 11$)
Serum immunoglobulins					
IgG (mg/100 ml)	532	515	NT	796	270-1010
IgA (mg/100 ml)	36	50	NT	125	2-78
IgM (mg/100 ml)	144	109	NT	87	36-106
Total leucocytes (per mm^3)	4000	5500	7200	9500	5652-10992
Total lymphocytes (per mm^3)	1760	3190	5328	3230	3433-7517
Lymphocytes subsets (%)					
OKT 11	NT	NT	NT	72.8	68.0-81.8
OKT 3	33.9	52.5	NT	70.2	54.4-73.0
OKT 4	22.8	32.2	NT	35.3	32.3-48.7
OKT 8	12.5	16.7	NT	25.2	18.8-32.6
OKIa 1	39.8	19.6	NT	16.1	10.3-21.9
Blastogenesis ($\text{cpm} \times 10^{-3}$)					
PHA	NT	NT	190	NT	58-187
Con A	NT	NT	160	NT	55-158
PWM	NT	NT	56	NT	20-66
PHA skin test	NT	$\pm$	+	NT	

*Mizuguchi (1983).

NT, not tested ; m, month ; y, year.

lymphocytes and high number of OKIa1 positive lymphocytes as in the patients reported by Girot et al. (1983). Although megaloblastic anemia, excessive excretion of orotic acid, lymphopenia and decreased number of OKT3 positive lymphocytes were corrected at 8 month-old after 5 months uridine therapy (Fig. 1, Table 1), a skin test for PHA (10 μ g) was weak positive and challenge with dinitrochlorobenzene was negative. To study the effect of uridine on lymphocytes in vitro, we cultured lectin-stimulated PBL and LCL in the presence of 10 to 1000 μ M uridine at 1 year and 2 month-old after 11 months of uridine therapy. As shown in Tables 2 and 3, the incorporation of leucine into PHA-, ConA- and PWM- stimulated PBL and into LCL were increased in the patient by the addition of uridine, although that in control remained unchanged. The incorporation of thymidine in patient's LCL increased in the presence of 10 μ M uridine, although that in control's LCL did not (Table 3).

Fig. 1. Clinical course and treatment.

DISCUSSION

There are conflicting reports whether or not hereditary orotic aciduria involves an immune deficiency. The cases whom Girot et al. (1983) reported had lymphopenia, low number of OKT3 positive lymphocytes, a decrease in T-cell-mediated lymphocytolysis and an absence of PWM-induced plasma cell generation, although the case reported whom by Becroft et al. (1984) had no immunological abnormalities. Our case had lymphopenia and low number of OKT3 positive lymphocyte before the uridine therapy. We found uridine supplement increased the incorporation of leucine in lectin-stimulated PBL and LCL in vitro. These results suggest that uridine supplement might increase the important factors in immune response, then protect patients with this disease from severe infection. Since both the case of Huguley et al. (1959) and the case of Fox et al. (1969) had severe infection when they stopped nucleotide intake, continuous uridine supplement should be needed in order to maintain normal immune response.

Uridine supplement increased the incorporation of thymidine in patient's LCL. This result might be correlated with the megaloblastic change in erythroblasts.

TABLE 2. *Effect of uridine on the ^3H -leucine incorporation of PBL stimulated by PHA, ConA and PWM*

Responder (1.25×10^5 cells)	Uridine (μM)	^3H -leucine incorporation (cpm)		
		PHA	ConA	PWM
Patient	0	—	—	768 $\pm$ 164
	0	+	+	1,884 $\pm$ 56
	10	+	+	9,048 $\pm$ 384
	100	+	+	11,661 $\pm$ 167
	1000	+	+	11,487 $\pm$ 103
Control 1	0	—	—	486 $\pm$ 24
	0	+	+	4,796 $\pm$ 369
	10	+	+	4,931 $\pm$ 483
	100	+	+	4,753 $\pm$ 236
	1000	+	+	5,047 $\pm$ 384
Control 2	0	—	—	1,100 $\pm$ 16
	0	+	+	4,759 $\pm$ 101
	10	+	+	4,856 $\pm$ 189
	100	+	+	5,035 $\pm$ 136
	1000	+	+	4,279 $\pm$ 61

Values are means $\pm$ S.E.

TABLE 3. *Effect of uridine in EB virus-transformed B cell line*

Responder (1×10^5 cell)	Uridine (μ M)	3 H-leucine incorporation (cpm)	3 H-thymidine incorporation (cpm)
Patient's LCL	0	$5,751 \pm 183$	$101,900 \pm 1,570$
	1	$6,287 \pm 208$	$116,256 \pm 1,775$
	10	$17,013 \pm 890$	$215,016 \pm 5,179$
	100	$17,856 \pm 190$	$143,242 \pm 2,516$
	1000	$16,523 \pm 755$	$124,871 \pm 2,791$
Control's LCL	0	$30,205 \pm 494$	$218,478 \pm 3,579$
	1	$29,688 \pm 672$	$220,093 \pm 2,689$
	10	$32,677 \pm 1,285$	$216,356 \pm 1,631$
	100	$28,596 \pm 221$	$192,664 \pm 2,793$
	1000	$30,603 \pm 2,331$	$184,302 \pm 8,661$

Values are means $\pm$ S.E.

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