α -L-Iduronidase Activity in Established Lymphoblastoid Cells from Patients with Hurler and Scheie Syndromes Transformed by Epstein-Barr Virus

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MINAMI, R., SUZUKI, M., KUDOH, T., SATO, S., OYANAGI, K., NAKAO, T., SUKEGAWA, K. and ORII, T. *α -L-Iduronidase Activity in Established Lymphoblastoid Cells from Patients with Hurler and Scheie Syndromes Transformed by Epstein-Barr Virus.* Tohoku J. exp. Med., 1977, 122 (4), 393-396 — α -L-Iduronidase activity was determined in established lymphoblastoid cells, which were transformed in vitro by Epstein-Barr virus, of lymphocytes-rich cell populations isolated from peripheral blood of patients with Hurler and Scheie syndromes. α -L-Iduronidase activities in established lymphoblastoid cells from patients were undetectable, while activities of control subjects were clearly detected. These results suggest that established lymphoblastoid cells are useful for the enzymatic study of genetic mucopolysaccharidoses. ——— α -L-iduronidase; established lymphoblastoid cells; Epstein-Barr Virus; Hurler and Scheie Syndromes

It has been demonstrated that Epstein-Barr virus (EBV) can transform human lymphocytes into continuously growing lymphoblastoid cells in vitro, and that these cultured cells can be established into lymphoblastoid cell lines (Katsuki and Hinuma 1976). No report has so far been published in which lymphoblastoid cells were used for the enzymatic study of genetic mucopolysaccharidoses. This work is to determine enzyme activities of several lysosomal hydrolases in lymphoblastoid cells from patients with Hurler and Scheie syndromes, and normal subjects.

MATERIALS AND METHODS

Patients. Patient 1 (Hurler syndrome) was a 15-month-old female with severe growth and psychomotor retardation, coarse facial feature, corneal clouding, severe skeletal deformities, hepatosplenomegaly, umbilical hernia and numerous episodes of bronchopneumonia. Urinary excretion of acid mucopolysaccharides was 200.0 mg/liter (expressed as hexosamine). Patient 2 (Scheie syndrome) was a 16-year-old male with mild growth and slight psychomotor retardation, coarse facial feature, corneal clouding, mild skeletal deformities, hepatomegaly and umbilical hernia. Urinary excretion of acid mucopolysaccharides was 33.6 mg/liter.

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Establishment of lymphoblastoid cell lines by EBV. The lymphocytes of heparinized peripheral blood were separated by gradient centrifugation with Ficoll-Conray. After the lymphocyte pellet was washed twice with medium RPMI 1640 containing 20% fetal calf serum with 100 $\mu\text{g/ml}$ streptomycin and 100 IU/ml penicillin, cells were resuspended in medium to give 1.2×10^6 cells/ml. To 0.8 ml lymphocytes suspension (10^6 cells/ml), 0.2 ml of EBV suspension, which was a culture fluid of B95-8 established lymphoblastoid cells originated from marmoset monkey (Miller and Lipman 1973), was added. The lymphocytes cultures were incubated at 37°C and half volume medium was changed every 3 or 4 days. The lymphocytes were transformed within several weeks and lymphoblastoid cells were established. These established lymphoblastoid cells were subcultured every 4 to 5 days, and given rise to continuously growing cells (Katsuki and Hinuma 1976).

Preparation for enzyme assay. Each lymphoblastoid cell suspension culture was centrifuged, and the lymphoblastoid cell pellet was washed twice with 0.9% NaCl. To the cell pellet was added an aliquot of ice cold distilled water and was treated with an insonator for 2 min. The sonically disrupted suspension was used for the enzyme assay.

Leucocytes were obtained by the same method as previously described by Orii et al. (1976).

Assay of enzymes. α -L-Iduronidase activity was determined in a manner similar to that described by Liem and Hooghwinkel (1975) using phenyl- α -L-iduronide as substrate which was synthesized by the method of Friedman and Weissmann (1972). Incubation mixtures consisted of 25 μl of 5 mM phenyl- α -iduronide, 50 μl of each enzyme preparation and 25 μl of 0.4 M formate buffer (pH 3.5) containing 0.4 M NaCl. The reaction mixture was layered with 20 μl of toluene and incubated for 18 hr at 25°C, then extracted three times with 100 μl , 100 μl and 200 μl of toluene and to the pooled extracts 300 μl of 0.05 M NaOH was added.

Phenol was determined by addition of Folin-Ciocalteu reagent (400 μl of a freshly prepared dilution of 1 part reagent and 2 parts water) and 300 μl of 12% Na_2CO_3 . After the color of phenol was developed at 37°C for 20 min, absorbance was read at 660 m μ .

Other lysosomal hydrolases in enzyme preparation were assayed in the following incubation mixtures. β -D-Gactosidase: 0.1 ml of enzyme preparation, 0.5 ml of 0.8 mM 4-methyl umbelliferyl (4-MU)- β -galactopyranoside (Koch-Light) in 0.1 M citrate phosphate buffer, pH 4.0; N-acetyl- β -D-glucosaminidase: 0.1 ml of enzyme preparation, 0.5 ml of 1 mM 4-MU-N-acetyl- α -D-glucosaminide in 0.1 M citrate phosphate buffer, pH 4.0; α -D-mannosidase: 0.1 ml of enzyme preparation, 0.5 ml of 1 mM 4-MU- α -D-mannopyranoside in 0.1 M citrate phosphate buffer, pH 4.5. Each incubation was carried out at 37°C for 60 min. The reaction was stopped with 3 ml of 0.25 M glycine buffer, pH 10.7.

The fluorescence was read immediately in a Hitachi spectrophotofluorometer (model MPF-4) with excitation wave-length 365 m μ and emission wave-length 450 m μ . Arylsulfatase B was assayed according to Baum et al. (1959). Protein was assayed as described by Lowry et al. (1951).

RESULTS

The levels of lysosomal hydrolases assayed in leucocytes and established lymphoblastoid cells are shown in Tables 1 and 2. α -L-Iduronidase activity in established lymphoblastoid cells, as well as in leucocytes, from Hurler syndrome was undetectable. In Scheie syndrome, α -L-iduronidase activity was found to be deficient in established lymphoblastoid cells and was markedly reduced in leucocytes. The other lysosomal hydrolases assayed in both established lymphoblastoid cells and leucocytes from patients showed activities within normal range.

TABLE 1. *Acid-hydrolase activities in leucocytes*

	Enzyme activity per mg protein			
	α -L-Iduronidase (nmoles/18hr)	β -D-Galactosidase (nmoles/hr)	N-Acetyl- β -D glucosaminidase (nmoles/hr)	Arylsulfatase B (nmoles/hr)
Hurler Syndrome	0	334	1134	147
Scheie Syndrome	12	136	1288	81
Control Mean	185	284	1265	108
Range	105~371 (n=10)	167~417 (n=10)	460~2829 (n=10)	32~231 (n=10)

TABLE 2. *Acid-hydrolase activities in established lymphoblastoid cells*

	Enzyme activity per mg protein			
	α -L-Iduronidase (nmoles/18hr)	β -D-Galactosidase (nmoles/hr)	N-Acetyl- β -D glucosaminidase (nmoles/hr)	α -D-Mannosidase (nmoles/hr)
Hurler Syndrome	0	68	525	162
Scheie Syndrome	0	43	573	113
Control 1	13	47	464	58
2	19	51	616	152
3	10	44	537	83
4	26	121	691	173

DISCUSSION

As an *in vitro* model, the cultured skin fibroblast has been used for underlying biochemical defects in genetic mucopolysaccharidoses. In our experiments, α -L-iduronidase activities of established lymphoblastoid cells from Hurler and Scheie syndromes, transformed by EBV, were clearly distinguished from those of control subjects. Therefore, established lymphoblastoid cells, although transformed by EBV, express genetic metabolic defects in patients.

As compared with cultured skin fibroblasts, established lymphoblastoid cells transformed by EBV have the following advantages: Only 2-3 ml of peripheral blood are required. The culture cell line is established within several weeks. No alteration nor mutation of the cells occurs during culture or reculture (Kennedy 1976).

With the development of the techniques for the establishment and maintenance of lymphoblastoid cells transformed by EBV, an alternate cell culture system would be available for study of not only genetic mucopolysaccharidoses but also other inborn errors of metabolism.

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