Hexose Uptake and Transport in Polymorphonuclear Leukocytes from Patients with Glycogen Storage Disease Ib

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ABSTRACT. Neutrophil functions and glucose metabolism are known to be impaired in glycogen storage disease (GSD) Ib patients. The uptake of nonmetabolizing glucose analogues into polymorphonuclear leukocytes (PMN) of GSD Ib patients was studied. 2-Deoxyglucose (2-DOG) and 3-O-methylglucose are transported across the cell membrane by facilitated diffusion mediated by the glucose transporter. Because 2-DOG is phosphorylated within the cell, its uptake rate reflects hexose transport as long as phosphorylation is not rate-limiting. These conditions prevail only at low 2-DOG concentrations. Transport of 5 μ M 2-DOG into GSD Ib patient PMN was found to be similar to controls (4.3 ± 0.5 and 4.65 ± 1.77 pmol/min $\times 10^6$, respectively). In contrast, 2-DOG uptake at high concentrations (2 mM) decreased by 70% in patient PMN compared with control cells (0.17 ± 0.06 and 0.51 ± 0.11 nmol/min $\times 10^6$, for patients and controls, respectively). Transport of 3-O-methylglucose (a glucose analogue that does not undergo intracellular phosphorylation) was not different in patient PMN compared with controls (1.86 ± 0.53 and 2.19 ± 0.30 nmol/min $\times 10^6$, respectively). Hexose monophosphate shunt activity in PMN of GSD Ib patients at a glucose concentration of 2 mM was 43% of control values, whereas at 10 μ M it was similar to controls. Taken together, these results suggest that the defect in glucose uptake and metabolism found in GSD Ib patient PMN is due to an impairment in hexose phosphorylation rather than in a reduction in the transmembrane glucose transport activity. (*Pediatr Res* 28: 19–23, 1990)

Abbreviations

GSD, glycogen storage disease
PMN, polymorphonuclear leukocyte
3-O-MG, 3-O-methylglucose
2-DOG, 2-deoxyglucose
G6P, glucose-6-phosphate
HMP, hexose-monophosphate
KRP, Krebs Ringer phosphate
FMLP, formyl-methionyl-leucyl-phenylalanine

GSD Ib (McKusick 23222) is a metabolic disease caused by a defect in the translocase for G6P in the liver microsomal membrane (1, 2). As a result, hepatic glucose-6-phosphatase in GSD

Ib patients is nonfunctional *in vivo*. GSD Ia (McKusick 23220) is caused by a deficiency of the G6Pase activity. Patients with GSD Ib are often neutropenic and prone to recurrent infections; otherwise, the clinical features of GSD Ib are indistinguishable from GSD Ia. There is increasing evidence of functional defects in the neutrophils of GSD Ib patients that limit their ability to destroy invading microorganisms. Defects in random and direct cell migration, extra phagocytotic respiration, chemotaxis bactericidal activity, and calcium mobilization in response to FMLP was found in the patient neutrophils (3–10, and Kilpatrick L, Garty BZ, Lunquist KF, Stanley CA, Baker L, Douglas SD, Korchak HM, personal communication).

A decreased rate of 2-DOG uptake into PMN collected from GSD Ib patients was previously demonstrated (6, 8, 10). 2-DOG is widely used as a nonmetabolizable glucose analogue to study the transport of hexose in various cell types. 2-DOG enters the cell by way of facilitated transport similar to glucose, and it is phosphorylated within the cell to 2-deoxyglucose-6-phosphate. The phosphorylated analogue is not metabolized further and is trapped within the cell, since it can no longer traverse the cell membrane (11–13). Thus, uptake of 2-DOG reflects transport as long as the hexokinase activity does not become rate-limiting, a condition that is obtained only at low 2-DOG concentrations (14).

Another analogue of glucose is 3-O-MG. This analogue is transported across PMN membrane by the glucose transporter, but, unlike 2-DOG, it is not further metabolized within the cells. Therefore, it can be used for direct assessment of hexose transport across PMN cell membrane (15, 16).

Our study suggests that the defect in glucose uptake into GSD Ib neutrophils is derived from impairment in hexose phosphorylation rather than a direct alteration in transmembrane glucose transport.

MATERIALS AND METHODS

Patients. GSD Ib was diagnosed in liver biopsies by a high latency (*i.e.* low activity) of glucose-6-phosphatase in fresh homogenate and normal activity in disrupted microsomes (17). The six GSD Ib patients suffered from numerous infections. The clinical and therapeutic data of the patients and one GSD Ia patient are summarized in Table 1. Studies of PMN were performed with preparations at an initial cell count of 600 to 1500 cells/mm³.

Cell Preparation. PMN from patients with GSD Ib and from healthy donors were separated from heparinized venous blood by the Ficoll-Hypaque centrifugation method (6). Briefly, one volume of fresh blood was mixed with two volumes of 3% dextran in 0.85% NaCl. After one hour at 4°C, the supernatant was layered on top of Ficoll-Hypaque (Pharmacia Inc., Piscataway, NJ) at a volume ratio of 6 mL of cell suspension to 3 mL

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Table 1. Laboratory data of patients studied

	GSD Ib patients						GSD Ia TY
	LS	AA	NT	RA	BM	SS	
Age (y)	13	4	3	3/12	2	5	3
Lactic acid (mM)	3.1	3.7	2.9	4.6	1.8	4.5	2.8
Uric acid (mg/dL)	4.7	7.0	5.7	4.5	7.5	7.3	7.0
Cholesterol (mg/dL)	98	128	173	112	142	129	230
Serum glutamic oxaloacetic transaminase (IU)	54	67	120	33	35	42	62
Serum glutamic pyruvic transaminase (IU)	46	40	100	35	12	46	71
Total leukocytes (cells/mm ³)	3100	5700	5500	4800	3500	4100	13 000
PMN (% of total leukocytes)	24	27	15	7	6	17	28

of Ficoll. After centrifugation for 30 min at 5°C at 800 × *g*, the pellet was collected for PMN preparation. Contaminating red cells were removed by the addition of 6 mL distilled H₂O for 20 s, and isotonicity was restored by the addition of 2 mL 3.5% NaCl.

Cells were suspended in KRP buffer, counted in a hemocytometer, and adjusted to 30–40 × 10⁶ cells/mL. The final preparations contain >85% PMN in both patients and control samples.

Transport Studies of Glucose Analogues into PMN. 2-DOG transport. A total of 1–2 × 10⁶ purified PMN suspended in 45 μL KRP were incubated for 15 min at 37°C in a 4.5 mL round-bottomed mini scintillation vial (14). 2-DOG uptake was initiated by adding 15 μL KRP buffer containing 1-³H-2-DOG [1.48 MBq/mL (40 μCi/mL)] in concentrations indicated in figure legends. Uptake (linear up to 5 min) was stopped after 2 min by the addition of 2 mL cold 0.85% NaCl containing 0.3 mM phloretin, and 0.1 μM HgCl₂. The samples were centrifuged at 3000 × *g* for 3 min and the pellets were washed twice by centrifugation with 2 mL cold 0.85% NaCl. The washed pellets were examined for radioactivity in instagel scintillation fluid, using a Packard tricarb spectrometer (Packard Instrument Co., Inc., Downers Grove, IL).

3-O-MG Transport. Transport of 3-O-MG during a 20 s time period was measured in a similar way to 2-DOG transport. Uptake was initiated by adding 15 μL KRP buffer containing 3-O-MG [5.5 MBq/mL (150 μCi/mL)] at the concentrations indicated in the figure legends. The subsequent procedure was the same as described for 2-DOG transport.

Phosphorylation of 2-deoxyglucose. 10⁶ PMN were incubated at 37°C in a final volume of 60 μL containing 2 mmol/L 1-³H-2-DOG [7 KBq/mL (0.2 μCi)]. Cells were washed as described for 2-DOG. The final pellets were suspended in 1 mL water and boiled for 5 min in a water bath. Subsequently, denatured protein was removed by centrifugation. The supernatant was applied to a column of Dowex-I-Cl-resin (10 cm × 1 cm) as described before (11). The column was washed with 10 mL water to elute free 2-DOG and 10 mL 0.5 mol/L HCl to remove 2-deoxyglucose-6-phosphate. Samples of 1 mL from each fraction were measured for radioactivity.

Activity of the HMP Shunt. The activity of the HMP shunt was determined by a modification of the method described by Newburgh *et al.* (18). Briefly, PMN were suspended in KRP (10⁶ cells/mL) with [1-¹⁴C]-glucose [18 KBq/mL (0.5 μCi/mL)] at the concentrations indicated in Table 4, and 1 mmol/L methylene blue. Incubation at 37°C for 1 h was performed in sealed tubes with a central well containing filter paper impregnated with 100 μL of hyamine-hydroxide. At the end of the incubation period, 0.5 mL of H₂SO₄ (2 mol/L) was added to stop the reaction and to release ¹⁴CO₂ from the solution. The tubes were subsequently shaken for 30 min. The filter paper was removed and placed in vials filled with a toluene-based scintillation fluid, and radioactivity was determined in a tricarb spectrometer.

Statistical Analysis. Data are presented as the mean ± SD. For each assay, not less than three separate observations were made in independent experiments. The *t* test was used to compare the

mean value of control assays with those of GSD Ib patients (*n* = 6).

Materials. D-1-³H-2-Deoxyglucose, 3-O-methyl-D-[1-³H]glucose, and 1-¹⁴C-glucose were purchased from Amersham Co., Buckinghamshire, UK. All other chemicals were purchased from Sigma Chemical Co., St. Louis, MO.

RESULTS

The rate of 2-DOG and 3-O-MG uptake by PMN of GSD Ib and GSD Ia patients under saturating concentrations, as compared with controls, is demonstrated in Table 2. 2-DOG transport in PMN from individuals in different age groups (from 5 d to 40 y old) was the same (9). Uptake of 2-DOG is reduced to 30% in the PMN of GSD Ib patients as compared with GSD Ia patients or controls. By contrast, no significant difference was observed in the rate of 3-O-MG transport between patient and control PMN.

Figure 1 shows zero trans entry (all sugar analogues were initially only outside the cell) of 3-O-MG and 2-DOG as a function of concentration. Both 2-DOG and 3-O-MG are taken up into PMN by facilitated diffusion. The uptake of both sugars follows saturation type kinetics, with *K_m* values of 0.6 mM and 5 mM and *V_{max}* of 0.51 ± 0.11 and 2.19 ± 0.3 nmol/min × 10⁶ for 2-DOG and 3-O-MG, respectively. The different kinetic constants observed could be due to different affinities of the

Table 2. 2-deoxyglucose and 3-O-methylglucose uptake in human PMN of GSD Ia and GSD Ib patients and normal controls*

	2-deoxyglucose uptake nmol/10 ⁶ cells × min	3-O-methylglucose uptake nmol/10 ⁶ cells × min
Controls	0.51 ± 0.11 (21)†	2.19 ± 0.30 (7)†
GSD Ia patients	0.31 ± 0.12 (4)†	1.8 ± 0.4 (4)†
<i>p</i> value‡	NS	NS
GSD Ib patients		
NT	0.14 ± 0.02 (9)§	1.39 ± 0.42 (4)§
LS	0.08 ± 0.03 (6)§	1.76 ± 1.02 (6)§
AA	0.22 ± 0.01 (3)§	2.44 ± 0.62 (3)§
SS	0.18 ± 0.06 (3)§	ND
BM	0.18 (2)§	ND
RA	0.26 (1)§	ND
Overall mean Ib patients	0.17 ± 0.06 (6)§	1.86 ± 0.53 (3)§
<i>p</i> value¶	<0.05	NS

* The initial rates of 2-DOG and 3-O-MG transport were measured as described in Materials and Methods at saturating concentrations of substrate (2-DOG at 2 mM and 3-O-MG at 15 mM).

† Number of controls and GSD Ia patients.

‡ *t* test comparing mean of control values with mean of GSD Ia patients.

§ (), number of experiments performed.

|| ND, not determined.

¶ *t* test comparing mean of control values with mean of GSD Ib patients.