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METABOLIC CORRECTION OF FUCOSIDOSIS LYMPHOID CELLS BY GALAPTIN-α-L-FUCOSIDASE CONJUGATES[1]

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SUMMARY. To determine if isolated galaptin, an endogenous galactoside-binding lectin, could serve as a transport vehicle of therapeutic agents to cells, galaptin and α-L-fucosidase were coupled using glutaraldehyde. The conjugates were incubated with α-L-fucosidase-deficient, EBV-immortalized lymphoid cells from a fucosidosis patient. Conjugates were effectively bound and internalized by the cells in a lactose inhibitable manner. Internalization of conjugate resulted in the reduced accumulation of α-L-fucosyl-N-acetylglucosaminylasparagine, a glycopeptide that accumulates in cells of fucosidosis patients, to levels found in lymphoid cells from a healthy individual. Thus, galaptin-α-L-fucosidase conjugates may be useful for enzyme replacement therapy of fucosidosis. The concept of using galaptin as a transport vehicle may be applied to the delivery of other compounds to cells bearing galaptin receptors.

Lysosomal storage diseases are a group of over thirty individual lethal inborn errors of metabolism (1,2). In most of the diseases, there is deficient synthesis of an active form of a lysosomal enzyme. This results in substrate accumulation within lysosomes leading to cell and organ dysfunction, and premature death. Unfortunately, effective treatment of humans with lysosomal storage diseases by enzyme replacement or by any other means has not been successful (1,3). It is now recognized that for enzyme replacement therapy to be effective, improvements are needed in the delivery of enzyme to cells and to sites of substrate accumulation within cells (3-5).

This communication reports preliminary data concerning a novel delivery vehicle for enzymes to cells. Galaptin is a galactoside-binding lectin that is found in many vertebrates, including humans (6). Lactosaminoglycans that are putative receptors for galaptin (7) are present at the surface of lymphoid cells (8). EBV-immortalized lymphoid cells from a normal donor were found to have 2.1 x 10^7 galaptin receptors/cell with an apparent Ka of 1.3 x 10^9 M^{-1} (manuscript in preparation). We reasoned that galaptin may be an effective targeting agent of enzymes to lymphoid cells when conjugated to enzymes. To test this idea, we prepared conjugates of galaptin to α-L-fucosidase (α-L-fucoside fucohydrolase, EC 3.2.1.51). α-L-Fucosidase was chosen because it has been shown that fucosidosis lymphoid cells lack a mechanism for significant uptake of enzyme from

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Abbreviations: Fuc-GlcNAc-Asp, α-L-fucosyl-N-acetylglucosaminylasparagine; BSA, bovine serum albumin; PBS, Dulbecco's phosphate buffered saline.

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culture medium (unpublished observations). Fucosidosis is a lysosomal storage disease characterized by accumulation of fucoglycoconjugates due to deficient activity of α-L-fucosidase (9,10). Moreover, assay of Fuc-GlcNAc-Asp, a glycopeptide that accumulates in the tissues and fluids of fucosidosis patients (11-16), can be conveniently carried out.

MATERIALS AND METHODS B lymphoid cell lines used were B-142 derived from a healthy individual and JT from a fucosidosis patient. The establishment of cell lines from peripheral blood, detailed background information, and routine culture conditions have been described (17,18). Galaptin was purified from human spleen (19) and α-L-fucosidase from human omentum (20). Fuc-GlcNAc-Asp was isolated from lymphoid cells as described (21). Identity of the glycopeptide was determined by thin layer chromatography on silica in $CHCl_3$:CH_3OH:NH_4OH (8:16:3) and in $CHCl_3$:CH_3OH:H_2O (60:35:8).

Galaptin was alkylated with [^{14}C]-iodoacetamide (22) prior to preparation of polygalaptin or with unlabeled iodoacetamide (22) prior to preparation of galaptin-α-L-fucosidase conjugate. Alkylation was necessary to eliminate the thiol requirement for maintenance of carbohydrate binding activity. For polymerization of galaptin, 120 μl of 1% glutaraldehyde were slowly added to 1.0 mg galaptin in 1.0 ml of 0.2M NaCl\8.0mM PO_4\0.1M lactose, pH 7.3. The solution was stirred for 17 h at 4 °C. Then 100 μl of 1.0M Tris, pH 7.3, were added and the sample was incubated for 6 h at 4 °C. Bovine serum albumin (10 mg) was added and the sample was dialyzed against 0.5M NaCl\10.0mM Tris, pH 7.3. The polymerized galaptin was then re-purified by affinity chromatography on lactosyl-Sepharose (19). The lactose eluate was made 1.0 mg BSA/ml; it was concentrated by centrifugal filtration and stored at 4 °C. In some cases, the product was made to 50% glycerol and stored at -20 °C, occasionally with prior adsorption of polygalaptin to DEAE Sephacel. The preparation of galaptin-α-L-fucosidase conjugates was as above with the starting galaptin solution also containing 0.3 mg α-L-fucosidase\0.1M L-fucose. Carbohydrate binding activity was checked by hemagglutination assay (23) and α-L-fucosidase activity by assay with 4-methyl-umbelliferyl-α-L-fucoside as substrate (17). One unit of α-L-fucosidase activity is 1 nmole of substrate hydrolyzed per hr. Sepharose 6B and DEAE Sephacel were products of Pharmacia Inc. [1-^{14}C]Iodoacetamide, 17.9 mCi/mmole, was obtained from ICN Pharmaceuticals Inc. [6-^{3}H]L-Fucose, 90 μCi/mmole, was obtained from Dupont-NEN Inc.

RESULTS During the conjugation of galaptin with a protein under the conditions described, it was anticipated that the conjugates formed might have polymerized galaptin as part of the macromolecular complex. Therefore, the feasibility of using galaptin as a delivery vehicle of a test compound to cells would depend upon the ability of not only galaptin but also polygalaptin to bind to cells in a carbohydrate specific manner. The data in Table I show that both galaptin and polygalaptin bind to lymphoid cells in a lactose-inhibitable manner. Moreover, lymphoid cells bound over 15-fold more polygalaptin than galaptin when the same amounts of galaptin and polygalaptin were incubated with cells.

Table I

Binding of Radiolabelled Galaptin and Polygalaptin to Fucosidosis Lymphoid Cells

Addition	Amount Bound to Cells	
	μg	%
Polygalaptin (14 μg)	1.60	8.0
Polygalaptin (14 ug)+ 50mM lactose	0.05	0.3
Polygalaptin (20 μg)	3.08	15.4
Polygalaptin (20 μg)+ 50mM lactose	0.29	1.5
Galaptin (20 μg)	0.19	1.0
Galaptin (20 ug)+ 50mM lactose	0.02	0.1

JT lymphoid cells, 10^6 in 0.1 ml of RPMI 1640 medium containing 0.5% BSA and the indicated additions, were incubated at 4 °C for 90 m. The cells were centrifuged at 250 x g at 4 °C for 10 m. Media were removed; the cell pellets were washed twice with PBS containing 0.5% BSA, and cell-bound radioactivity was determined. Specific activities of [^{14}C]polygalaptin and [^{14}C]galaptin were 800 dpm/μg.

Table II

Binding of Galaptin-α-L-Fucosidase Conjugate to Fucosidosis Lymphoid Cells

Addition	Fucosidase Activity[a]		
	Cells	Media	Total
Experiment 1			
Galaptin-α-L-Fucosidase (4u[b])	0.416	3.560	3.976
Galaptin-α-L-Fucosidase (4u) + 50mM lactose	0.012	4.240	4.252
α-L-Fucosidase (4u)	0.005	4.040	4.045
α-L-Fucosidase (4u) + 50mM lactose)	0.010	4.140	4.150
No additions to fucosidosis cells	0	0	0
50mM Lactose	0	0	0
Experiment 2			
Galaptin-α-L-Fucosidase (4u)	0.320	2.880	3.200
Galaptin-α-L-Fucosidase (4u) + 50mM lactose	0.007	3.680	3.687
Galaptin-α-L-Fucosidase (8u)	0.567	6.080	6.647
Galaptin-α-L-Fucosidase (8u) + 50mM lactose	0.020	7.680	7.700
Galaptin-α-L-Fucosidase (12u)	0.733	9.760	10.493
Galaptin-α-L-Fucosidase (12u) + 50mM lactose	0.027	11.580	11.547
No additions to fucosidosis cells	0	0	0
50mM lactose	0	0	0

[a]nmoles substrate hydrolyzed/hour.
[b]u = units.
JT lymphoid cells, 10^6 in 0.1 ml of RPMI 1640 medium containing 0.5% BSA and the indicated additions, were incubated at 4 °C for 90 m. The cells were centrifuged at 250 x g at 4 °C for 10 m. Media were removed and saved. The cells were washed twice with PBS containing 0.5% BSA and were extracted with 25 ul of 1% Triton X-100. Cell extracts and media were assayed for α-L-fucosidase activity.

To determine the suitability of galaptin as a delivery vehicle, conjugates of galaptin and α-L-fucosidase were prepared and administered to cultured lymphoid cells deficient in α-L-fucosidase activity. In this system, association of conjugate with cells is conveniently measured by assaying for α-L-fucosidase activity.

The specificity of cell binding of galaptin-α-L-fucosidase conjugates and of purified α-L-fucosidase was determined. Binding was carried out at 4 °C to minimize turnover and/or recycling of putative cell surface receptors during the binding interval. The results are summarized in Table II. In two separate experiments, cells incubated with conjugate only (4 enzyme units) bound 10.5% and 10.0% of the total (cells + medium) enzyme activity recovered; whereas cells incubated with conjugate in the presence of lactose bound 0.3% and 0.2%. Thus, binding of conjugate to cells was almost completely inhibited by lactose. Cells incubated with free α-L-fucosidase (4 units) in the absence or presence of lactose bound 0.1% and 0.2% of the total enzyme activity recovered. Thus, binding of enzyme to cells via galaptin receptors was 50 to 100-fold more efficient than binding of enzyme to endogenous enzyme receptors. The amount of enzyme bound to cells increased when increasing amounts of conjugate were incubated with the cells (Table II, Exp 2). Thus, binding of enzyme was dependent upon the concentration of conjugate in the incubation medium.

Whether or not the galaptin-α-L-fucosidase conjugate could be internalized by cells was determined as described in Table III. Briefly, cells were allowed to bind conjugate at 4 °C followed by internalization at 37 °C. At given time intervals, a culture was transfered to 4 °C, divided into two aliquots, and media were removed. The two cultures were incubated at 4 °C in PBS, one with and one without lactose. The inability to remove cell-associated enzyme by treatment with lactose was considered evidence of conjugate internalization. The data in Table III show that cell-associated conjugate progressively became resistant to removal by lactose when cells were incubated at 37 °C from 0 h to 4 h.

Table III

Internalization of Galaptin-α-L-Fucosidase Conjugate By Fucosidosis Lymphoid Cells

Addition	Time at 37 °C (h)	Enzyme activity[a] Medium	+ Lactose Cells	+ Lactose PBS-BSA	-Lactose Cells	-Lactose PBS-BSA
Experiment 1						
Galaptin-α-L-	0	5.12	0.040	0.592	0.212	0.108
Fucosidase	1	5.92	0.088	0.464	0.194	0.096
Conjugate	2	6.56	0.128	0.448	0.180	0.112
(6 units)	4	5.12	0.212	0.320	0.236	0.094
Experiment 2						
Galaptin-α-L-Fucosidase Conjugate (18 units)	0	17.28	0.090	0.550	0.385	0.180
	4	14.88	0.255	0.220	0.315	0.120
α-L-Fucosidase	0	20.92	0.010	0.005	0.020	0.005
(18 units)	4	19.68	0.015	0.005	0.015	0.005

[a]nmoles substrate hydrolyzed/hour.

JT lymphoid cells, $2X10^6$ in 0.2 ml of RPMI 1640 medium containing 0.5% BSA and the indicated additions, were incubated at 4 °C for 90 m. Then 0.4 ml medium containing 0.5% BSA was added to each tube. The cells were transferred to 37 °C. After 0, 1, 2, or 4 h, the cells were centrifuged at 250 x g at 4 °C for 10 m. Medium was removed and saved. The cells were washed twice with PBS containing 0.5% BSA and divided into two aliquots of 10^6 cells in 0.1 ml of PBS containing 0.5% BSA with and without 50 mM lactose. The cells were then incubated at 4 °C for 90 m to allow displacement of surface-bound conjugate, followed by centrifugation. The PBS-BSA supernates were removed and saved. Cells were washed and were extracted with 25 μl of 1% Triton X-100. Cell extracts, media, and PBS-BSA supernates were assayed for α-L-fucosidase activity.

Table IV

Effect of Galaptin-α-L-Fucosidase Conjugate Internalization on Hydrolysis of [^{3}H]Fuc-GlcNAc-Asp in Fucosidosis Lymphoid Cells

Cell Type	Addition	[^{3}H]Fuc-GlcNAc-Asp CPM	% of control
		Experiment 1[a]	
Fucosidosis (JT)	None (Control)	72840	100
Normal (B142)	None	7740	11
		Experiment 2	
Fucosidosis (JT)	NONE	87380	100
(JT)	Galaptin-α-L-Fucosidase (191 μg, 432 units[b])	3310	4
(JT)	Galaptin-α-L-Fucosidase (191 μg, 432 units) + 50mM Lactose	90580	104
(JT)	α-L-Fucosidase (432 units)	85200	98
		Experiment 3	
Fucosidosis (JT)	None	85970	100
(JT)	Galptin-α-L-Fucosidase (191 μg, 432 units)	14070	16
(JT)	Galaptin-α-L-Fucosidase (191 μg, 432 units) + 50mM Lactose	71570	83
(JT)	Polygalaptin (191 μg) + α-L-Fucosidase (432 units)	68230	79

[a][^{3}H]Fuc-GlcNAc-Asp was determined after 24 h incubation with [^{3}H]fucose.
[b]nmoles substrate hydrolyzed/hour.

Lymphoid cells (10^6/ml) in RPMI 1640 medium containing 10% fetal calf serum were incubated with [^{3}H]fucose (10 uCi/ml) for 24 h at 37 °C. Cells were harvested by centrifugation at 250 x g for 10 m at 4 °C and were washed twice with RPMI 1640 medium containing 0.5% BSA. Aliquots of $3x10^6$ cells were suspended in 0.3 ml of RPMI 1640 containing 0.5% BSA and the indicated additions. The cells were incubated at 4 °C for 90 m to allow binding of conjugate. The cells were then diluted with 2.7 ml of RPMI 1640 + 0.5% BSA and were incubated at 37 °C for 20 h to allow internalization of conjugate. The cells were harvested for isolation of [^{3}H]Fuc-GlcNAc-Asp as described in Materials and Methods.

Additionally, when α-L-fucosidase was substituted for conjugate, virtually no enzyme activity was bound or internalized. Conjugate but not free enzyme was capable of being bound and internalized by cells.

The fucosidosis lymphoid cells accumulated Fuc-GlcNAc-Asp 9-fold more than lymphoid cells derived from a healthy individual (Table IV, Exp. 1). This corroborated a previous investigation (16). To determine if galaptin-α-L-fucosidase conjugate could reduce the accumulation of Fuc-GlcNAC-Asp in fucosidosis lymphoid cells, cells were metabolically labeled with [^{3}H]L-fucose followed by incubation with conjugate, and assayed for content of [^{3}H]Fuc-GlcNAc-Asp (Table IV). In two experiments, cells treated with conjugate exhibited reduced accumulation of the glycopeptide to 4% and 16% of untreated cells. Cells treated either with conjugate in the presence of lactose, or with α-L-fucosidase, or with an unconjugated mixture of galaptin and α-L-fucosidase were not appreciably different from untreated cells. Therefore, the ability of conjugate to reduce the cellular accumulation of glycopeptide was dependent upon the binding of covalently linked galaptin and α-L-fucosidase to a lactose-inhibitable receptor. Additionally, treatment with conjugate reduced the accumulation of glycopeptide in fucosidosis lymphoid cells to a level similar to that found in lymphoid cells derived from a healthy individual (Table IV).

DISCUSSION The data presented demonstrate that galaptin is an effective transport vehicle of α-L-fucosidase to fucosidosis lymphoid cells and to the intracellular site of accumulated endogenous substrate. This suggests that galaptin-α-L-fucosidase conjugate may be useful for enzyme replacement treatment of fucosidosis. To our knowledge enzyme replacement therapy of fucosidosis has not been attempted.

The binding and uptake of galaptin-α-L-fucosidase by lymphoid cells was inhibited by lactose. This indicates that many cell types bearing galaptin receptors at the cell surface may be a potential target. N-Acetyllactosamine-containing structures commonly occur in complex carbohydrates and are found on a variety of cell types. This may be especially useful in treating fucosidosis, since cells of all organs of fucosidosis patients apparently accumulate storage material (10). Additionally, since galaptin-α-L-fucosidase conjugate is composed of proteins of human origin, the conjugate may be weakly or nonimmunogenic *in vivo*.

The use of galaptin as a transport vehicle may not be limited to α-L-fucosidase targeting and may be applied to other lysosomal enzymes and lysosomal storage diseases. Another potential use for galaptin in disease treatment could be as a transport vehicle of normal gene constructs to cells with mutant genes. However, it needs to be determined if galaptin-delivered genes can intracellularly express a functional protein product. Fucosidosis lymphoid cells and the isolated human gene for α-L-fucosidase (24, 25) constitute an *in vitro* system for testing this idea.

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