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Enzyme Replacement with Recombinant β-Glucuronidase in the Newborn Mucopolysaccharidosis Type VII Mouse

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ABSTRACT. β-Glucuronidase injected i.v. into newborn mucopolysaccharidosis VII mice was cleared from the circulation in less than 1 h and taken up by tissues in a distribution corresponding to the location of the mannose 6-phosphate receptor. One h after a 3.5-mg/kg β-glucuronidase injection, β-glucuronidase levels were equal to or greater than normal in every organ examined with the exception of the brain, where 31% normal activity was present. Enzyme was detectable histochemically in the major sites of pathology for mucopolysaccharidosis VII including bone, brain, heart, and fixed tissue macrophages. The half-life of recombinant β-glucuronidase activity in various organs of injected mucopolysaccharidosis VII mice was 1.5 to 4.5 d. These studies show that recombinant β-glucuronidase administered to newborn mice reaches the sites of clinically important storage in murine mucopolysaccharidosis VII. (*Pediatr Res* 34: 837–840, 1993)

Abbreviations

MPS, mucopolysaccharidosis
β-gluc, β-glucuronidase
BMT, bone marrow transplantation
Man 6-P, mannose 6-phosphate

The mucopolysaccharidoses are inherited disorders, each caused by a deficiency of one of the lysosomal enzymes necessary for the degradation of glycosaminoglycans. Affected patients have glycosaminoglycan accumulation in lysosomes and progressive organ dysfunction. A murine model of MPS type VII that lacks β-gluc (EC 3.2.1.31) shares many clinical, biochemical, and pathologic features with human MPS VII (Sly syndrome) (1, 2) and allows controlled therapeutic studies of genetically identical animals.

Infusion of deficient lysosomal enzyme has been proposed as a therapy for lysosomal storage diseases (3). However, sufficient quantities of lysosomal enzymes with the recognition markers needed for receptor-mediated endocytosis have not been available, except for glucocerebrosidase in Gaucher disease. The availability of large quantities of phosphorylated recombinant β-gluc (4) made experimental enzyme replacement therapy for murine MPS VII feasible. Newborn MPS VII mice have only small amounts of lysosomal storage (2) and enzyme therapy instituted early in life might prevent further accumulation of storage and arrest disease progression. We present evidence that i.v. administered β-gluc is taken up by many tissues in the MPS VII mouse and its cellular distribution is similar to that reported for the Man 6-P/IGF-II receptor responsible for receptor-mediated endocytosis and targeting of lysosomal enzymes to lysosomes (5, 6).

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MATERIALS AND METHODS

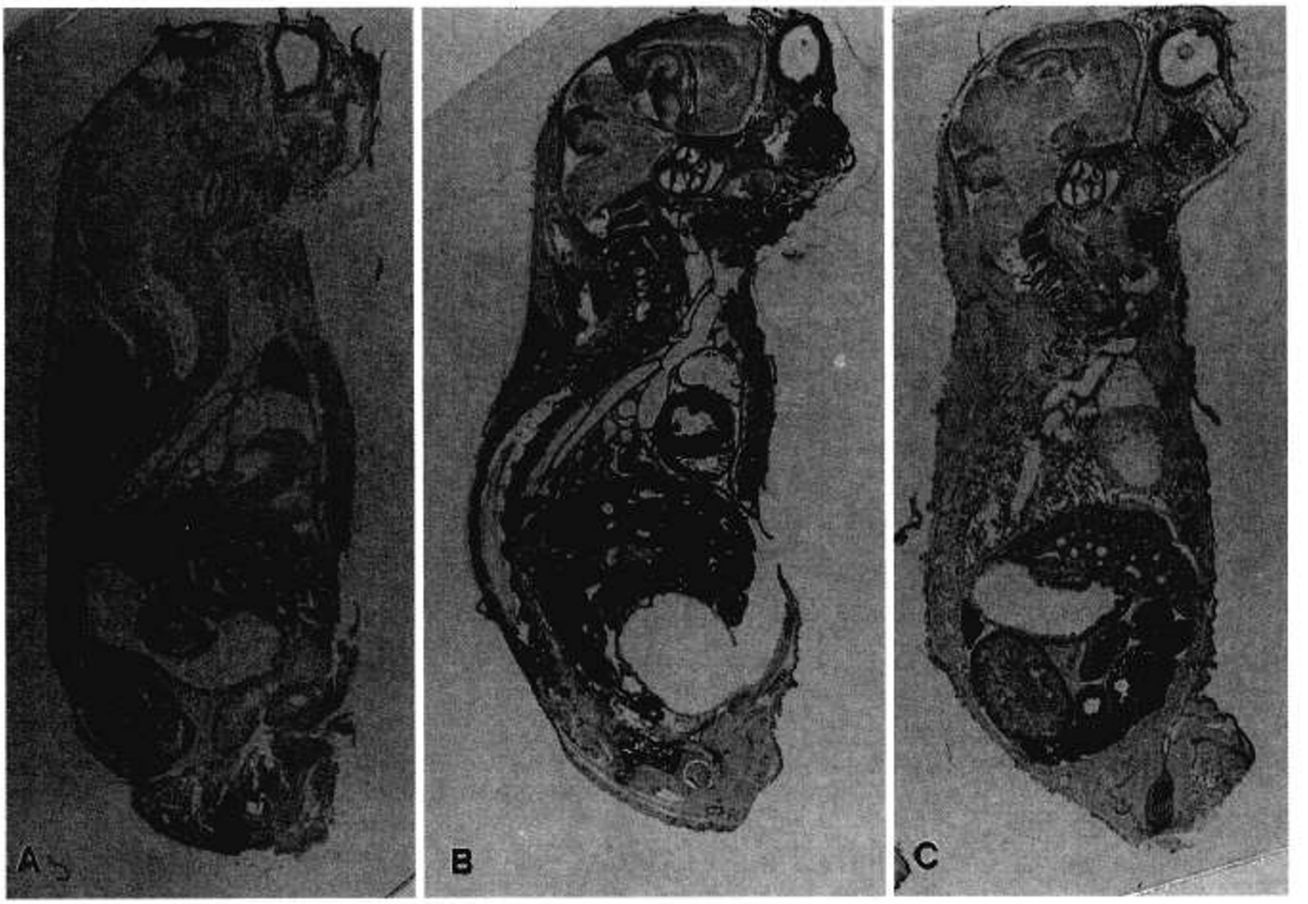
Twenty-eight thousand units of recombinant β-gluc (approximately 3.5 mg/kg) prepared as previously described (4) and dissolved in 100 μL of buffer containing 10 mM Tris (pH 7.5), 150 mM NaCl, and 1 mM β-glycerol phosphate were injected into the superficial temporal vein of three 1-d-old MPS VII pups. Controls included two homozygous normal and two MPS VII 1-d-old pups injected with diluent buffer. All experiments were performed with the highest standards of humane animal care. The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Mice were killed 1 h after the injection. For histochemical study, tissues were immersed in 0.25% sucrose as a cryopreservative or OCT (Miles, Elkhart, IN) and frozen in liquid nitrogen–cooled isopentane. Ten-μm-thick tissue sections were stained for β-gluc activity using a histochemical method (7). In two enzyme-injected MPS VII pups and a normal and an MPS VII control, sagittal sections of the whole animal were studied.

β-gluc activity was assayed (8) in multiple tissues from an enzyme-injected MPS VII pup and compared with the enzyme levels in age-matched untreated MPS VII and normal pups as previously described (1). To determine β-gluc half-life in MPS VII pups during the 1st wk of life, six mice were injected with 28 000 U of β-gluc at 1 d of age and one mouse was killed at 24, 48, 72, 120, and 168 h postinjection. Homogenates of liver, kidney, spleen, lung, heart, brain, and residual carcasses were assayed for β-gluc activity and protein concentration (9). Log sp act *versus* time after injection was plotted to calculate enzyme half-life. *r* values were determined for each of the curves by the least squares method.

RESULTS

In the MPS VII control pup receiving buffer alone, β-gluc activity was not identified histochemically or biochemically in any tissue (Fig. 1*A*). In contrast, 1 h after enzyme injection, MPS



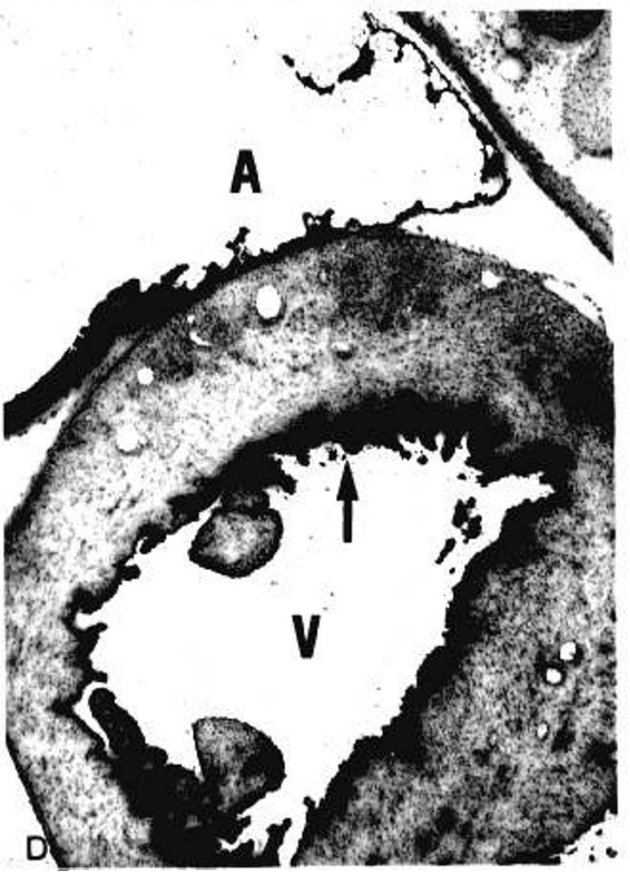


Fig. 1*D*

Fig. 1*E*

VII pups had β-gluc activity in multiple sites (Fig. 1*B*, Tables 1 and 2). Although the intensity of staining was less overall in the normal control pup, the distribution of β-gluc activity was similar in many sites to that in the MPS VII pup after enzyme injection (Fig. 1*C*, Table 2).

The fixed tissue macrophage system in MPS VII pups readily took up injected β-gluc. Other sites, including the endocardium (Fig. 1*D*) and bone (Fig. 1*E*), also showed intense enzyme activity after injection. In the stomach and small intestine, the lamina propria contained abundant, diffuse enzyme activity, and in the small intestine, villus epithelial cells had activity in circumscribed supranuclear zones. However, crypt epithelium contained no activity. In the CNS of injected MPS VII pups, β-gluc activity was present in vessels, meninges (Fig. 1*F*), and the connective tissue core of the choroid plexus. Although we could not identify enzyme activity in the CNS neurons, ganglia neurons in the peripheral nervous system had a small amount of β-gluc activity.

One h after the single injection, approximately 70% of the injected enzyme was recovered in homogenates of organs and carcasses of MPS VII pups and less than 1% remained in the plasma. With the exception of the CNS, which contained 31% normal activity, normal or greater than normal levels of β-gluc activity were present in all organs examined (Table 2). The half-life of the injected recombinant β-gluc during the 1st wk of life was different in each tissue and was longest in the brain (Table 2).

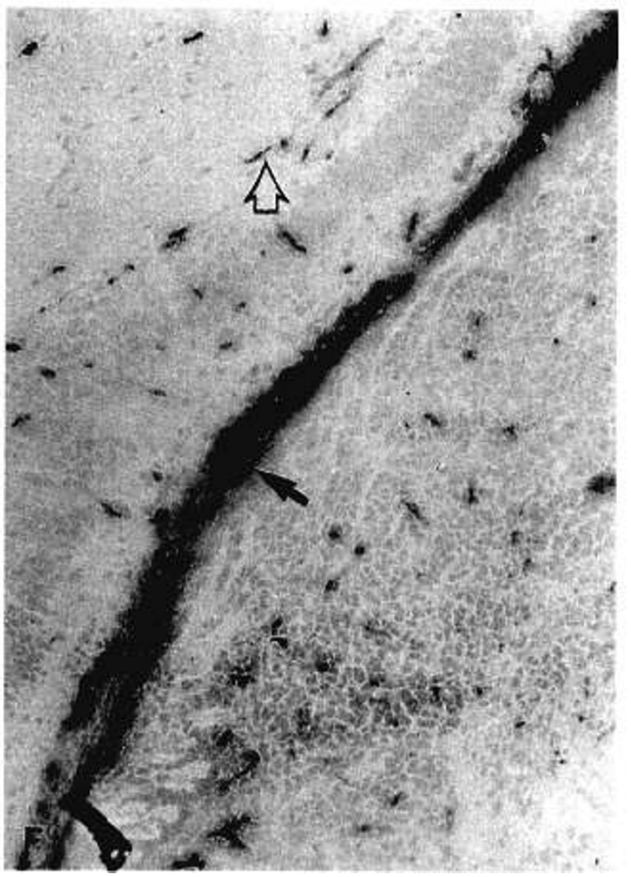


Fig. 1*F*

DISCUSSION

Recombinant β-gluc is taken up by many tissues and cell types in the newborn MPS VII mouse. Presumably, rapid clearance of enzyme from plasma is mediated by mannose and Man 6-P receptors. Participation of the Man 6-P receptor in enzyme uptake is suggested by the correlation of the distribution of enzyme with the receptor's previously reported location in fetal and newborn rodents (5, 6, 10). Sites reached after i.v. injection of 28 000 U of β-gluc, which is 3 to 5 times the amount of enzyme present in a homozygous normal pup, include the brain,

Table 1. *Comparison of tissue β-gluc histochemical activity**

	MPS VII mouse 1 h after enzyme injection	Normal mouse, no enzyme
Bone		
Osteoid	+++	++
Perichondrium	+(focal)	+
Chondrocytes	++(focal)	++
Bone marrow	++	++
Nervous system		
Meninges	++	+
Choroid plexus	+++	+(focal)
Vessels	+	+
Neurons (CNS)	−	−
Satellite cells, ganglia	++	+
Ganglion cells	+	+(focal)
Nerve trunks	+	+
Heart		
Endocardium	+++	−
Myocardium	+	−
Other tissues		
Liver	+++	++
Spleen	+++	++
Stomach		
Columnar epithelium	−	++
Lamina propria	++	+
Small intestine		
Villus epithelium	+++	+++
Crypt epithelium	−	−
Lamina propria	+++	−
Muscularis	−	−
Kidney	+++	+
Adrenal	++	+
Brown fat	++	+
Skin		
Epidermis	−	−
Dermis	+	+
Lung		
Smooth muscle	++	−
Columnar epithelium	−	+
Great vessels	++	−
Skeletal muscle		
Fibers	−	−
Vessels	++	+
Eye sclera	+++	−

* +++, Intense staining; ++, moderate staining; +, weak staining; −, no enzyme detected histochemically.

Fig. 1. *A-C*, Sagittal sections of whole-mounted pups, stained for β-gluc activity. Positive tissues stain bright red. *A*, An MPS VII control pup contains no tissue enzyme activity. *B*, An MPS VII pup 1 h after β-gluc injection shows intense staining in many sites including the liver, bone, and heart. There is also enzyme in the CNS, particularly in the choroid plexus stria vascularis, meninges, and vessels. *C*, A homozygous normal control pup has less intense staining for β-gluc except in the intestine. *D-F*, Tissues from an MPS VII pup 1 h after β-gluc injection, stained for β-gluc activity. *D*, The endocardium (*arrow*) of the atrium (*A*) and ventricle (*V*) is decorated by intense staining for β-gluc. *E*, Bones in all sites examined have intense β-gluc activity in the osteoid, osteoblasts, and osteocytes. The marrow also contains enzyme activity. *F*, The brain has β-gluc activity in the meninges (*black arrow*) and vessels (*open arrow*) (naphthol ASBI-β-glucuronide, *A-C* 4.7×, *D* 51×, *E* 128×, and *F* 128×).

Table 2. *Percent of normal β-gluc activity in MPS VII pups after enzyme injection and β-gluc half-life during 1st wk of life*

Organ	Percent normal		Half-life in h (*r* value)
	1 h	1 wk	
Heart	2300	14.3	34 (0.979)
Liver	1300	39.8	50 (0.994)
Kidney	209	13.2	72 (0.939)
Lung	137	2.7	36 (0.982)
Spleen	99	7.8	84 (0.936)
Brain	31	1.4	108 (0.924)

bone, heart, and fixed tissue macrophage system. Thus, β-gluc is targeted to sites that exhibit clinically important lysosomal storage in MPS VII.

The relative level of β-gluc activity in the CNS achieved by a single injection in the newborn MPS VII mouse is substantially higher than that produced by neonatal BMT (11). Thus, the CNS may respond better to early enzyme therapy than to BMT performed immediately after birth. Achieving β-gluc levels of 31% normal in the CNS is significant because enzyme levels as low as 2% and 6% have been shown to markedly reduce morphologic evidence of lysosomal storage in the liver and spleen, respectively (7). Although enzyme replacement may prove to be beneficial as a sole form of therapy in MPS (as is clearly the case in Gaucher disease), replacement therapy might also be used as a bridge to BMT. With this approach, exposure of newborn MPS VII mice to radiation, which causes growth retardation and focal dysplasia of the CNS (11), would be avoided. Therapeutic levels of infused enzyme could prevent lysosomal glycosaminoglycan accumulation until mice are old enough to safely undergo BMT.

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