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REVIEW

Animal Models for Lysosomal Storage Disorders

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Abstract—The lysosomal storage disorders (LSD) represent a heterogeneous group of inherited diseases characterized by the accumulation of non-metabolized macromolecules (by-products of cellular turnover) in different tissues and organs. LSDs primarily develop as a consequence of a deficiency in a lysosomal hydrolase or its co-factor. The majority of these enzymes are glycosidases and sulfatases, which in normal conditions participate in degradation of glycoconjugates: glycoproteins, glycosaminoproteoglycans, and glycolipids. Significant insights have been gained from studies of animal models, both in understanding mechanisms of disease and in establishing proof of therapeutic concept. These studies have led to the introduction of therapy for certain LSD subtypes, primarily by enzyme replacement or substrate reduction therapy. Animal models have been useful in elucidating molecular changes, particularly prior to onset of symptoms. On the other hand, it should be noted certain animal (mouse) models may have the underlying biochemical defect, but not show the course of disease observed in human patients. There is interest in examining therapeutic options in the larger spontaneous animal models that may more closely mimic the brain size and pathology of humans. This review will highlight lessons learned from studies of animal models of disease, drawing primarily from publications in 2011-2012.

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Lysosomal storage disorders (LSD) may develop as a consequence of a deficiency of an enzyme/hydrolase or its co-factor/activator [1]. In some cases, the defect may be in a transport protein or in the post-translational modification of an enzyme, which leads to its inactivation or premature degradation. In most of these disorders the end result is similar: there is intra-lysosomal accumulation of incompletely metabolized substrates, by-products of cellular turnover [2]. Over the last century, the clinical manifestations, biochemical, and/or molecular basis of distinct LSDs have been characterized. These developments have enabled the introduction of a facile means of diagnostic confirmation and in some cases the subsequent introduction of therapy. However, there remains incomplete understanding of disease mechanisms. This article will review lessons drawn from investigations of animal models of the LSD, including those identified in nature (i.e. spontaneous) and generated by recombinant genetic techniques; the latter are mainly mouse models [3].

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BIOCHEMISTRY AND PATHOPHYSIOLOGY

The majority of patients with LSD are often diagnosed after the onset of symptoms, and the number of postmortem examinations has declined over the years. Thus, most of the recent characterizations of tissue alterations and downstream biochemical/molecular changes have been undertaken in cognate animal models. This is illustrated by studies in the "knock-in" mouse model for mucolipidosis II (ML-II), a neurometabolic lysosomal enzyme trafficking disorder caused by the loss of mannose 6-phosphate (M6P) signals on lysosomal enzymes. Most lysosomal enzymes are routed to the lysosome and taken up by the M6P receptor. In ML-II, deficiency of a hexameric ($\alpha_2\beta_2\gamma_2$) *N*-acetylglucosamine (GlcNAc)-1-phosphotransferase complex limits the formation of M6P residues on *N*-linked oligosaccharides of lysosomal enzymes [4]. Other routing mechanisms have been identified including lysosomal integral membrane protein 2 (LIMP2) mediated transport of β-glucocerebrosidase (the enzyme deficient in Gaucher disease) and sortilin, a multifunctional receptor implicated in targeting several

proteins to the lysosome, including sphingolipid activator proteins (prosaposin and G_{M2} activator protein), acid sphingomyelinase, and cathepsins D and H [5].

Examination of affected ML-II mouse brains revealed the expression and proteolytic processing of several distinct lysosomal proteins (e.g. α-L-fucosidase, β-hexosaminidase, α-mannosidase, or Niemann–Pick C2 protein) were significantly impacted by the loss of M6P, e.g. in contrast to those enzymes, e.g. β-glucocerebrosidase, which reach lysosomes independently of this targeting mechanism. As a consequence, various substrates accumulate, including fucosylated N-glycans, G_{M2}- and G_{M3}-gangliosides, cholesterol, and bis(monoacylglycero)phosphate [6]. Prominent astrogliosis and the accumulation of organelles and storage material in focally swollen axons were observed in the cerebellum, wherein there was marked loss of Purkinje cells. An increased neuronal level of the microtubule-associated protein 1 light chain 3 and the formation of p62-positive neuronal aggregates were also seen, which indicate an impairment of constitutive autophagy.

Neurodegenerative features are seen in several LSDs, and those associated with G_{M2}-ganglioside accumulation (e.g. G_{M1}- and G_{M2}-gangliosidosis, galactosialidosis) have been shown in various animal models to be associated with meganeurite formation and abnormal dendritogenesis [7]. In these disorders, neuronal cell death may be due to a combination of factors, including disruption of endoplasmic reticulum stress responses, defects of axonal transport and neuronal–glial interactions, as well as secondary inflammatory reactions and activation of autophagy [8]. Interestingly, in the case of Tay–Sachs disease (β-hexosaminidase deficiency) the corresponding mouse model, which displays the biochemical and neuropathologic features, does not show the neurodegenerative course of disease observed in the infantile form of the human disorder [9]. This has been partly attributed to the presence of an alternative route for G_{M2}-ganglioside degradation in mice when compared to humans [10]. Thus, caution should always be exercised in studies of animal models [11].

In the early years of the XXth century, the focus of studies in LSDs was on the phenotypic delineation (i.e. clinical manifestations) and the biochemical characterization of the storage material; the latter explains the basis for prior classification of LSDs into gangliosidosis, mucopolysaccahridosis (i.e. glycosaminoglycan storage), oligosaccharidosis, etc. [12]. Subsequently, recognition of the block in catabolic pathways enabled identification of the responsible enzyme and identification of the corresponding molecular gene defect. Recently, the spotlight has shifted to investigations of associated cellular events, which has directed attention to defects of autophagy in several distinct LSDs.

Autophagy is a lysosomal mediated process for the degradation and recycling of various substrates and effete organelles [13]. Defects of autophagy have been implicated in several LSDs, including Niemann–Pick disease type C (NPC) and Pompe disease (acid α-glucosidase, GAA, deficiency) [14, 15].

NPC is a neurovisceral lipidosis caused by a defect in either the NPC1 protein (>95% of cases) or NPC2 (HE1) and an associated disturbance in the handling of cholesterol, among several other disruptions in cellular homeostasis [16]. NPC1 is a transmembrane protein, whereas NPC2 is a soluble (nonenzymatic) protein; the concerted action of these two distinct proteins enables lysosomal efflux of cholesterol. Marked accumulation of autophagosomes is a feature of NPC cells, attributed to the induction of autophagy through a beclin 1 (BECN1)- and lipid storage-dependent mechanism, associated with impairment in the clearance of autophagosomes due to inhibition of lysosomal protease activity [17]. Indeed, the intralysosomal accumulation of cholesterol is believed to create a positive feedback loop wherein autophagy induction exacerbates the disease via increased lipid storage [18]. Inhibition of autophagy has been shown to reduce cholesterol storage and restore normal lysosomal proteolysis in NPC1-deficient cells. Interestingly, β-amyloid metabolism has been shown to be altered in NPC, although there were specific differences in patterns of amyloid precursor protein (APP) degradation products between pharmacologically and genetically induced models [19]. This observation was attributed to complex interactions between APP metabolism and NPC-induced pathways [19].

In Pompe disease, large pools of autophagic debris can be found in skeletal muscle cells of both the GAA knockout (KO) mouse model and affected patients. Confocal microscopy of single muscle fibers stained for the lysosomal marker lysosomal associated membrane protein 1 (LAMP1) and an autophagosomal marker LC3 has revealed autophagic accumulation in virtually every type II fiber, even in young Pompe disease-affected mice [20]. In many fibers, the autophagic area localized mainly in the core and spread throughout the length of the fiber, with or without interruptions, which indicated not only were the lysosomes filled with undigested glycogen, but other materials were also backed up. Interestingly, therapeutic enzyme given to the Pompe mouse indicated oxidative type I muscle fibers responded to therapy much better than glycolytic type II muscle fibers despite the significantly higher glycogen burden in type I-rich muscles [20]. These observations suggest that the autophago-lysosome dysfunction may also interfere with cellular delivery of the recombinant enzyme and may ultimately influence therapeutic outcome.

Of further interest are studies of the neuropathological changes observed in mouse models of mucopolysaccharidosis (MPS) type I (α-L-iduronidase deficiency), III-A (sulfamidase deficiency), and III-B (α-N-acetylglucosaminidase deficiency). All of these types of MPS

are characterized by tissue storage of heparan sulfate (HS). Abnormally N-, 6-O, and 2-O heparan sulfated substrates were found in brain tissue from these affected mice, which may potentially alter HS-dependent cellular functions. Quantitative immunohistochemistry performed on brains from these animal models revealed several changes, including significantly increased lysosomal compartments, G_{M2}-ganglioside storage, neuroinflammation, decreased and mislocalized synaptic vesicle associated membrane protein (VAMP2), and decreased postsynaptic protein, Homer-1, in layers II/III-VI of the primary motor, somatosensory, and parietal cortex [21]. Neuroinflammation was confirmed by significantly increased monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), and interleukin-1α (IL-1α), using cytometric bead arrays. Brains from MPS III-A and III-B mice showed significantly more pronounced pathology than MPS-I (which can be associated with non-neuropathic forms, i.e. Hurler–Scheie and Scheie syndrome): in lysosomal storage, astrocytosis, microgliosis, and the percentage of 2-O sulfation of HS. A recent report underscores the complex changes seen in MPS, which also points to different neuropathogenic mechanisms predominating in distinct brain regions [22]. In the MPS-VII (β-glucuronidase deficiency) mouse model, transcriptome analysis revealed unexpected system and process alterations, such as up-regulation of the immune system with few inflammatory changes (a significant difference from the closely related MPS III-B model), down-regulation of major oligodendrocyte genes even though white matter changes are not a feature histopathologically, and a plethora of developmental gene changes [22].

PRECLINICAL TRIALS AND THERAPEUTIC OUTCOMES

Animal models of LSDs have been instrumental in establishing proof of therapeutic principle, by demonstrating substrate clearance from tissues, with associated improvement in outcomes including increased survival.

In Fabry disease (α-galactosidase A, AGAL deficiency), preclinical trials in the KO mouse model given recombinant AGAL showed delivery of the enzyme to target sites of pathology, including the heart and kidneys [23]. Subsequent trials in human Fabry disease (FD) patients revealed regular infusions of the recombinant enzyme resulted in the stabilization or improvement of renal function, when patients received treatment at an earlier stage of the disease process and did not have significant proteinuria [24]. Resolution of cardiomyopathy, particularly in those with significant left ventricular hypertrophy, has not been achieved, and cardiac complications remain a major source of morbidity and the most common cause of death among enzyme therapy-treated FD patients. It is likely that this is partly due to fibrosis, evident on cardiac magnetic resonance imaging with gadolinium as late-enhancement, particularly in the posterior wall of the heart [25]. Moreover, pathologic studies have shown the actual amount of glycosphingolipid storage in the heart of patients with FD represent a small proportion of its dry weight, which indicates other mechanisms contribute to development of cardiomyopathy.

Investigations of the FD KO mouse revealed the presence of mild hypertrophic cardiomyopathy with structural and functional alteration similar to that described for the early stages of human cardiomyopathies [26]. Molecular studies have revealed increased mRNA levels for the following natural compounds: atrial natriuretic factor (ANF), brain natriuretic peptide (BNP), plasminogen activator inhibitor-1 (PAI-1), connective tissue growth factor (CTGF), and thrombospondin 2 (TSP2). These changes are consistent with early stages of cardiac remodeling [26]. Enzyme therapy, using a single intravenous injection of 3 mg/kg agalsidase-beta, higher than the conventional dose given to human patients, was associated with a decrease in mRNA levels of ANF and BNP normalized to GAPDH. Also shown was a decrease in PAI-1 and CTGF mRNA levels, compared to untreated FD KO controls. However, there was no effect on the left ventricular hypertrophy, function, or heart rate. Perhaps, because treatment was only given once in the mice, whereas enzyme replacement therapy is designed for long-term administration (in humans, as an infusion once every two weeks, potentially lifelong).

In Pompe disease (PD), treatment with recombinant GAA has been associated with the resolution of cardiomyopathy in infantile patients. However, there can be persistent skeletal motor problems with age. This has raised some concern that M6P receptor targeting of Chinese hamster ovary cell (CHO)-derived enzyme, the currently available formulation, may be a sub-optimal option. As noted above, there was also concern about defects in autophagy possibly interfering with glycogen clearance in treated patients.

For decades, it was believed that targeting of recombinant lysosomal enzyme for use as therapy of LSDs required M6P residues for delivery to pathologic sites of storage. Recently, other options have been considered to overcome potential limitations associated with this approach. In one study, human GAA was fused to a Glycosylation-Independent Lysosomal Targeting (GILT) tag, which contains a portion of insulin-like growth factor II (IGF-II) [22]. The purpose was to create an active, chimeric enzyme with high affinity for the cation-independent M6P receptor (CI-MPR). In these studies, GILT-tagged GAA (BMN 701) was taken up by L6 myoblasts about 25-fold more efficiently than was recombinant (non-GILT) human GAA, and was significantly more effective in clearing glycogen from numerous skeletal muscle tissues in the PD mouse model [27]. This

enzyme formulation is currently in phase I clinical trials (ClinicalTrials.gov Identifier: NCT01230801).

Other therapeutic approaches, which remain investigational, include the use of pharmacological chaperones and gene therapy [28, 29]. Studies in various LSD animal models have given investigators reasons to be hopeful.

A major challenge in the treatment of LSDs is the presence of neurodegenerative features in certain subtypes. Several options have been examined, including direct injection of the relevant gene/protein into brain parenchyma and into the intra-techal space. Recently, intranasal administration of concentrated Aldurazyme® (laronidase) was shown to increase α-L-iduronidase (IDUA) activity, detected throughout the brains of MPS-I mice (IDUA deficient) [30]. Similarly, increased enzyme activity was also found following a single intranasal treatment with an adeno-associated virus (AAV) vector expressing human IDUA [30]. These results suggest that intranasal routes of delivery may be an alternative way to bypass the blood-brain-barrier to treat neuropathic forms of LSDs.

The majority of preclinical trials have been undertaken in the mouse model of LSDs [31]. Interestingly, not all mouse models, which bear mutations identical to that seen in human patients, express a phenotype that mimics the human condition [11]. The latter observations in certain models suggest species-specific differences in cellular metabolism and/or in the downstream mechanisms of disease and in putative mediators of pathology. Also, the complexity and brain size of humans may not be adequately reflected in affected mice, which have been shown to be responsive to multiple, including non-specific, therapies [29, 31]. Thus, there has been an increased interest in conducting experiments in large animal models.

One example of studies undertaken in a large animal model involved the Dachshunds homozygous for a null mutation in the *TPP1*, which encodes tripeptidyl-peptidase 1 (deficient in patients with late infantile neuronal ceroid lipofuscinosis, LINCL). Affected dogs recapitulate many of the features of disease in humans. Intrathecal (IT) TPP1 treatment reduced storage accumulation in many areas of the central nervous system [32]. Although there was no improvement in overall function, therapy attenuated the expected functional decline in TPP1 null Dachshunds. Further studies to optimize the dosing route and regimen to attenuate functional decline in this large animal model may provide insights into strategies applicable in humans.

Investigations undertaken in animal models of LSDs have provided significant insights into biochemistry and pathophysiology, in particular changes in gene expression profile during the early stages of the disease process prior to onset of clinical manifestations. Several disease mechanisms, including aberrant inflammatory responses and defects of autophagy, appear to be shared by distinct LSDs, which for some subgroups may partly explain the overlap in clinical features. Understanding of downstream molecular events may reveal potential therapeutic targets and enable optimal patient outcomes. These studies may also lead to identification of potential biomarkers, which can be used as surrogate endpoints in clinical trials. Shared disease pathways may imply that certain therapeutic strategies may have broad indications across several LSDs.

The majority of LSDs are associated with neurodegenerative features, and various therapeutic strategies are being explored to overcome the challenges presented by the blood brain barrier. There is active screening for small molecular agents, which can inhibit substrate synthesis and/or act as pharmacological chaperones to rescue mutant (misfolded) protein and thereby restore lysosomal function. Animal models have been engaged in these studies, to investigate drug delivery to selective sites of neuronal vulnerability in the brain. It is hoped these investigations will lead to the first success with treatment of some LSDs, such as Tay–Sachs disease, that currently are untreatable. Meanwhile, there is interest in expanding newborn screening programs to include inborn errors of metabolism resulting from lysosomal dysfunction. These endeavors have been partly promoted, because therapy is available for a subset, including Gaucher, Fabry, Pompe and MPS-I, -II and -VI. Early detection will enable the introduction of treatment, at an earlier stage in the disease process, when the potential for reversal may be greatest. As diagnostic confirmation, including prenatally, is available for most LSDs by biochemical and/or molecular assays, there are means to address recurrence risks in future pregnancies.

Animal models have been an invaluable resource in advancing the science of LSDs and the care of patients. As the focus shifts to studies of larger animal models, new avenues of investigations are anticipated to provide additional insights.

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