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Review article

Substrate deprivation therapy: a new hope for patients suffering from neuronopathic forms of inherited lysosomal storage diseases

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Abstract. Lysosomal storage diseases are a group of disorders caused by defects in enzymes responsible for degradation of particular compounds in lysosomes. In most cases, these diseases are fatal, and until recently no treatment was available. Introduction of enzyme replacement therapy was a breakthrough in the treatment of some of the diseases. However, while this therapy is effective in reduction of many somatic symptoms, its efficacy in the treatment of the central nervous system is negligible, if any, mainly because of problems with crossing the blood-brain-barrier by intravenously administered enzyme molecules. On the other hand, there are many lysosomal storage diseases in which the central nervous system is affected. Results of very recent studies indicate that in at least some cases, another type of therapy, called substrate deprivation therapy (or substrate reduction therapy) may be effective in the treatment of neuronopathic forms of lysosomal storage diseases. This therapy, based on inhibition of synthesis of the compounds that cannot be degraded in cells of the patients, has been shown to be effective in several animal models of various diseases, and recent reports demonstrate its efficacy in the treatment of patients suffering from Niemann-Pick C disease and Sanfilippo disease.

Keywords: Gaucher disease, genistein, lysosomal storage, miglustat, mucopolysaccharidoses, Niemann-Pick disease, Sandhoff disease, substrate deprivation therapy, Tay-Sachs disease.

Introduction

Among several thousands of genetic diseases, only a very small number can be treated. Several lysosomal storage disorders (LSDs) belong to this small group of treatable inherited diseases. Thus, LSDs became paradigms for developing new therapeutic strategies for genetic disorders. Apart from our knowledge on details of genetic and biochemical mechanisms of these disorders (Reuser and Drost 2006), the development of specific therapies of LSDs is being facilitated by the use of animal models, as many human diseases from this group have their equivalents in animals (Suzuki et al. 2003; Świtoński et al. 2004; Gieselmann 2006; Haskins 2007). In this mini-review we address the problem of treatment of neuronopathic forms of LSDs, and signalize comment on recent studies, which provide a possible solution to this problem.

Lysosomal storage diseases

LSDs are a group of 50 or so inherited diseases, caused by deficiency in one of enzymes involved, directly or indirectly, in degradation of various compounds (for a recent review, see Reuser and Drost 2006). Each LSD is caused by one or more mutations in a particular gene, which result in a significant decrease or abolishment of activity of a specific enzyme. This is manifested in accumulation of certain compounds (e.g. glycosaminoglycans, glycosphingolipids) in lysosomes. The resultant lysosomal dysfunction leads to cellular pathology, and then to changes in structure and function of tissues and organs. Although there

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are characteristic symptoms of each LSD, there is a high variability in severity of dysfunctions of particular organs and dynamics of the disease progress in individual patients.

Most LSDs are fatal disorders, with limited expected lifespan (in some cases as short as a few or several months). As in the case of all other inherited diseases, the treatment of LSDs has been found to be problematic. Nevertheless, an effective treatment of some LSDs has become available recently, and intensive studies are carried out to develop new therapies.

Treatment of somatic symptoms of LSDs

Bone marrow transplantation (BMT) was the first treatment modality used for LSDs. This method was found to be effective to some extent in some diseases (for example, in mucopolysaccharidosis type I) but not in others (for a review, see Neufeld and Muenzer 2001).

The break-through in the treatment of LSDs was the introduction of the enzyme replacement therapy (ERT). In this therapy, a recombinant enzyme is provided to patients whose organisms do not produce its natural active equivalent. Since the molecular signal of the uptake of lysosomal enzymes is known, the delivery of specifically modified recombinant enzyme molecules, administered intravenously, to target cells and their lysosomes became possible. The first disorder treated in this way was Gaucher disease, the most common in the group of LSDs. It is caused by a deficiency in glucocerebrosidase (a beta-glucosidase enzyme), which results in the storage of glucocerebroside in macrophages throughout the body (for a review, see Beutler 2006). The treatment of non-neuronopathic patients suffering from Gaucher disease with ERT appeared to be successful (Connock et al. 2006). It was possible not only to halt the progression of the disease, but also to correct most, if not all, symptoms.

Because of the spectacular success of ERT in Gaucher disease type 1 (with no involvement of the central nervous system), this type of therapy has been developed for some other LSDs. Thus, ERT for Fabry disease, Pompe disease, and mucopolysaccharidosis types I, II and VI is currently available (Brady 2006; Beck 2007). Many somatic symptoms of these diseases may be successfully treated with ERT, however, some problems remained unsolved. Intravenous administration of recombinant enzymes results in delivery of its molecules to most tissues, but not to the central nervous system due to the existence of the blood-brain-barrier. Therefore, neurological symptoms of LSDs cannot be successfully treated in this way.

Treatment of neuronopathic forms of LSDs

Since intravenous administration of the enzyme appears to be inefficient in treatment of the central nervous system in LSDs, studies on alternative therapeutic methods are being conducted. Although intrathecal administration of the enzyme was shown to reduce lysosomal storage of glycosaminoglycans in the brain and meninges of the canine model of mucopolysaccharidosis type I (Kakkis et al. 2004; Dickson et al. 2007), such a therapy would be perhaps problematic as a chronic treatment in humans, and especially in children. Other possible treatment strategies, which are currently under investigation, include gene therapy (Beck 2007), the stop codon read-through strategy (Brooks et al. 2006), the use of small synthetic chaperones (Pastores and Sathe 2006), and substrate deprivation therapy (SDT), also called substrate reduction therapy (SRD) (Beck 2007).

Gene therapy is considered a procedure that can be potentially used in the treatment of various genetic disorders, including LSDs (Beck 2007). However, it is still not available as an approved medical treatment.

The stop codon read-through strategy (Brooks et al. 2006) is based on the use of specific chemicals, which cause the misuse (with some frequency) of the stop codons during translation on ribosomes, and incorporation of an amino acid instead of translation termination. Thus, in the case of a nonsense mutation, which normally causes production of a truncated protein (usually with no biological activity), certain amounts of full-length, active polypeptide molecules can be produced. This may have a therapeutic effect in patients lacking a particular gene product. Such a strategy is still at the stage of laboratory experiments.

Point mutations in particular genes often lead to production of misfolded proteins. Such proteins are usually rapidly degraded by endoplasmic reticulum-associated proteases. Small molecules that interact with active centers of enzymes may, in some cases, act also as site-specific chaperones. They facilitate folding of mutant proteins, thereby accelerating their escape from the proteases. This causes maintenance of a higher level of residual enzyme activity (Pastores and Sathe 2006). The first experiments employing this therapeutic strategy for treatment of mice have recently been

reported, and results of those studies are encouraging (Fan and Ishii 2007). Nevertheless, it appears that particular chaperones may be specific only for misfolding of proteins caused by certain mutations.

SDT is based on the use of small molecules acting as inhibitors of synthesis of the compounds that cannot be degraded in lysosomes due to an enzymatic defect (Beck 2007). In principle, this should lead to restoration of the balance between synthesis and degradation of these compounds. If the inhibitory molecule is relatively small and can cross the blood-brain-barrier, it may act to correct the storage not only in somatic tissues but also in the central nervous system. Results of recent studies, summarized below, indicate that this option can be effective in the case of at least some neuronopathic forms of LSDs.

Gaucher disease

The first LSD treated with SDT was, again, Gaucher disease. The drug known as miglustat or Zavesca, i.e. 1,5-(butylimino)-1,5-dideoxy-D-glucitol), is an inhibitor of the synthesis of glucosylceramide and hence of subsequent metabolites. Miglustat has been approved as a drug for Gaucher disease type 1 (non-neuronopathic), as its administration results in reduction of somatic symptoms of this disorder (see, for example, Pastores et al. 2005; Aerts et al. 2006). Nevertheless, it was presumed that miglustat may be also effective in treatment of the neuronopathic form of Gaucher disease, i.e. type 3 of this disease. A recent article by Capablo et al. (2007) reports results of a combination of ERT with SDT in a patient suffering from Gaucher disease type 3, who developed neurologic deterioration and marked myoclonic epilepsy and dystonia. After 2 years of such a combined therapy, generalized tonic-clonic seizures decreased, while speech and general neurologic status improved. Therefore, it is tempting to speculate that SDT may be a potential treatment of the neuronopathic form of Gaucher disease, alone or in combination with ERT.

Tay-Sachs disease and Sandhoff disease

Tay-Sachs disease and Sandhoff disease belong to the group of gangliosidoses, severe diseases with dramatic neurological symptoms. Since in the biochemical pathway the synthesis of gangliosides is preceded by the synthesis of glucosylceramide, inhibition of production of the latter compound could potentially be employed in treatment of the diseases caused by defects in ganglioside degradation. Therefore, miglustat was considered an option for treatment of gangliosidoses.

In the studies on the use of SDT for Tay-Sachs and Sandhoff diseases, mouse models of these disorders were employed. It was demonstrated that in Tay-Sachs mice treated with miglustat, the accumulation of the ganglioside characteristic for this disease (called GM2) was prevented in the brain (Platt et al. 1997). In the case of the Sandhoff mouse model, treatment with miglustat caused a slower accumulation of gangliosides in the central nervous system (Jeyakumar et al. 2003). Therefore, it was proposed that SDT may be of therapeutic benefit in these diseases, especially in their juvenile and adult onset variants (Platt et al. 2003). It was also predicted that treatment of the infantile onset variants may be significantly more complicated (Platt et al. 2003). Indeed, a recent clinical study indicates that SDT of infantile Tay-Sachs patients could not prevent neurologic deterioration (Bembi et al. 2006).

Niemann-Pick C disease

Niemann-Pick disease type C is caused by impairment of regulation of intracellular lipid trafficking. This results in accumulation of free cholesterol and glycosphingolipids (including gangliosides) in many tissues, including brain (Liscum and Sturley 2004; Sturley et al. 2004), and causes severe neurological consequences.

Recent results indicate that implantation of neural stem cells in Niemann-Pick C mice extends life of these animals (Ahmad et al. 2007). However, this procedure was found to be only partially therapeutic, as the rate of weight gain and subsequent weight loss (resulting from neurodegeneration) in the treated mutant animals was not significantly different from untreated controls (Ahmad et al. 2007).

Because of ganglioside accumulation, miglustat was used for treatment of Niemann-Pick C disease in animal models. In those experiments, a reduction of ganglioside accumulation, delayed onset of neurological symptoms, and increased survival were observed in miglustat-treated animals (Zervas et al. 2001). Because of these positive effects, a randomized, controlled clinical trial was performed with patients suffering from Niemann-Pick C disease (Patterson et al. 2007). After 12 months of the study, positive neurological effects were observed in the miglustat-treated group in comparison to the control group. Particularly, horizontal saccadic eye movement velocity has improved ($P = 0.028$). Moreover, improvement in swallowing capacity and stable auditory acuity

were observed, and slower deterioration in ambulatory index was noted by Patterson et al. (2007). That was the first report demonstrating the efficacy of treatment of neurological symptoms in Niemann-Pick C patients. It indicates that SDT may be an effective therapy in treatment of neurological symptoms in patients suffering from this disease. Moreover, those results suggest that ganglioside accumulation, rather than cholesterol storage, is responsible for neurological symptoms of those patients (Erickson 2007).

Sanfilippo disease

Sanfilippo disease belongs to a group of LSDs called mucopolysaccharidoses (MPS), and is also known as mucopolysaccharidosis type III (or MPS III). This disease is caused by impairment of degradation of heparan sulfate, one of glycosaminoglycans (GAGs). This impairment may be due to dysfunction of 1 of 4 enzymes: heparan N-sulfatase, α-N-acetylglucosaminidase, acetyl-CoA:-glycosaminide acetyltransferase, or N-acetylglucosamine 6-sulfatase, causing Sanfilippo disease subtypes A, B, C, or D, respectively (Neufeld and Muenzer 2001). Since heparan sulfate accumulates in many tissues and organs, including brain, severe neurological symptoms occur in the patients.

Recent studies indicate that genistein, a chemical from the group of isoflavones (4', 5, 7-trihydroxyisoflavone or 5, 7-dihydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one), inhibits synthesis of GAGs in fibroblasts of patients suffering from various mucopolysaccharidoses (types I, II, IIIA and IIIB were tested) (Piotrowska et al. 2006). This inhibition appears to be due to a genistein-mediated inhibition of kinase activity of the Epidermal Growth Factor receptor (Piotrowska et al. 2006; Piotrowska, Jakóbkiewicz-Banecka, Węgrzyn, unpublished results). Apart from inhibition of GAG synthesis, a significant decrease in its storage was shown by biochemical methods and by electron microscopy when fibroblasts were cultured in the presence of genistein (Piotrowska et al. 2006).

The level of inhibition of GAG synthesis with rhodamine B, an inhibitor with an unknown mechanism of action, is similar to that observed in experiments with genistein (compare Piotrowska et al. 2006; Roberts et al. 2006). Importantly, Roberts et al. (2006) have demonstrated that in MPS IIIA mice treated with rhodamine B, a reduction in GAG storage decreases not only in somatic tissues, but also in brain. Moreover, very recent studies have provided evidence that treatment of MPS IIIA mice with rhodamine B results in improved behavior of the animals (Roberts et al. 2007).

Because of these encouraging results of experiments with genistein and rhodamine B, open-label, pilot clinical studies with children suffering from Sanfilippo disease types A and B were performed. A genistein-rich isoflavone extract (SE-2000, manufactured by Biofarm, Poland) was administered orally for 12 months. No significant side effects were observed. After one year of treatment, a statistically significant improvement in all tested parameters was demonstrated. Urinary GAG levels decreased ($P = 0.028$), hair morphology improved ($P = 0.012$), while cognitive functions improved markedly in 80% patients and stabilized in 20% patients ($P = 0.012$) (Węgrzyn et al. 2007; Piotrowska et al. submitted). Moreover, positive changes in behavior of treated children were observed. These included a decrease in hyperactivity and irritability, improvement in sleep disorders, better contact with children, and their improved concentration (Piotrowska et al. submitted). Improvements in cognitive and other mental functions are of special importance, as they indicate that higher brain functions that existed previously (at a pre-symptomatic stage of the disease) and were lost subsequently during development of the disease, can be restored (at least partially) when GAG accumulation is prevented and a quantity of already stored material is reduced.

Concluding remarks

Enzyme replacement therapy (ERT) has been introduced for several lysosomal storage diseases (LSDs) (Gaucher disease, Fabry disease, Pompe disease, and mucopolysaccharidoses types I, II and VI), and intensive studies are carried out to develop other therapies, but treatment of neurological symptoms of these diseases is still not possible. Recent clinical studies on patients suffering from Niemann-Pick C and Sanfilippo diseases demonstrate that substrate deprivation therapy (SDT) may be effective in humans, not only in delaying the appearance of symptoms or halting the progress of the disease (which was shown previously in studies on animal models), but also in reversion of at least some neurological symptoms. In fact, improvement of horizontal saccadic eye movement velocity in Niemann-Pick C disease provides

a first example of pharmacological correction of neurological symptoms of LSDs, and an increase in the scores of genistein-treated MPS III children in the psychological test indicates for the first time that SDT alone may lead to improved mental and cognitive functions in LSDs. These discoveries open new possibilities of studies on the use of SDT in treatment of various neuronopathic LSDs, and give a new hope for patients (and their families) suffering from as yet untreatable neuro-degenerative disorders.

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