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Pharmacotherapy of inborn errors of metabolism illustrating challenges in orphan diseases

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ABSTRACT

Orphan diseases (OD) have special challenges based on the rarity of the conditions. Mostly multicentre studies are required, controlled studies are difficult to perform. Based on the often chronic course of OD with slow progress the effect of therapeutic interventions is difficult to assess. Development and production of pharmaceutical substances for OD is difficult, time-consuming and sophisticated. Special incentives by the regulatory bodies like protocol assistance, long marketing exclusivity and reduced licencing fees encourage the development of orphan drugs.

Inborn errors of metabolism (IEMs) due to enzyme or transporter deficiencies are taken as an example for OD. Accumulation of substrates proximal to the deficient enzyme during catabolic episodes leads to autointoxication with acute onset of symptoms. IEMs due to transporter deficiency usually have a more stable, chronic course.

Therapeutic options are substrate reduction by diet or drugs, vitamin/cofactor supplementation, enzyme replacement, enzyme augmentation and transplantation of organs or cells.

Phenylketonuria (PKU) is the prototype of an IEM which can be successfully treated by diet. The outcome of hepatorenal tyrosinaemia type 1 was revolutionarized by substrate reduction using nitisinone (NTBC) which was discovered by chance. Lysosomal storage diseases are examples where enzyme replacement therapy is successful. Enzyme augmentation can be achieved in some IEM-patients with a mild phenotype (residual enzyme activity) by chaperones which stabilize the enzyme. Organ transplantation is an option in those patients who cannot be managed by drugs and/or diet. Bone marrow transplantation is successful in some patients where CNS-involvement occurs. The CNS cannot be reached by enzyme replacement therapy (blood–brain barrier).

While safety and efficacy of drugs for OD have been demonstrated pre-marketing, post-marketing surveys are often necessary to include more patients.

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1. Introduction

Orphan diseases (ODs) are rare diseases. The definition differs slightly from region to region: in Europe a condition is regarded as an OD if less than 1:2000 individuals are affected, in the USA <200,000 (1:1500), in Japan <50,000 (1:2500) persons are affected, in Australia less than 1:10,000 individuals are suffering from the disease. 6000–8000 different ODs are known today, about 30 million persons are estimated to suffer from OD in the European Union. 80% of OD have a genetic cause, hence are chronic diseases. 75% of OD affect children with 30% of OD patients dying before their 5th birthday (http://www.eurordis.org). This causes considerable distress to patients and their families. There are many unmet needs which led to the establishment of special frameworks for the advancement of OD (at the levels of research and development, approval and marketing) already in 1983 in the USA, in 1993 in Japan and finally in 2000 in the European Union. It still took several years until measures materialized at the national levels (Joppi et al., 2013; Facey et al., 2014). In Germany for example, a research report on measures to improve the state of health of people with rare diseases was solicited by the Ministry of Health in 2009, in 2010 the 'National Action League for People with Rare Diseases' (NAMSE) was founded by the Ministry of Health and the Ministry of Education and Research and only in 2013 the national action plan for people with rare diseases became operational. The aim is to improve care for patients with rare diseases and to point out the unmet needs.

Based on the small patient number in specific OD, research and development of pharmaceutical products (orphan drugs) is both difficult and expensive, to secure financial returns treatment costs per patient have to be high (cf Aronson, 2006). At the level of the European Union the 'European Medicines Agency' (EMA) offers scientific counselling (project assistance) free of charge, 10 years marketing exclusivity and reduced licencing fees. These measures led to the approval of 124 new orphan drugs from 2000 to 2015, another 1200 drugs for rare diseases have orphan drug designation (http://www.ema.europa.eu/ema/). It

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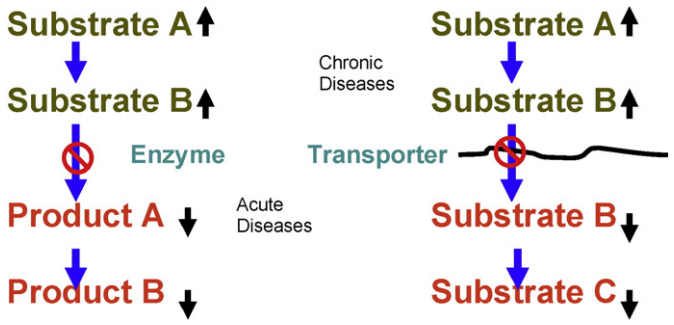

Fig. 1. Inborn errors of metabolism. An enzyme deficiency (left) leads to the accumulation of substrates of the deficient enzyme while its products are reduced. These diseases typically have an acute onset (triggered mostly by catabolism, 'intoxication'-type IEM). In a transporter deficiency (right) the substrate of the transporter accumulates in one cell compartment while it is low in another cell compartment. These diseases typically have a chronic course.

has been shown that OD development is economically feasible in this context (Meekings, Williams, & Arrowsmith, 2012). Differences exist regarding legislation and policies for OD development and the authorization process in different regions of the world (Franco, 2013).

As an example for OD this review will focus on inborn errors of metabolism (IEMs) and illustrate the specific challenges in this group of rare diseases.

2. Inborn errors of metabolism

In 1908, Sir Archibald Garrod paved the way for IEMs by giving a seminal lecture at the Royal College of Physicians in London where he for the first time described an IEM, called alkaptonuria. IEMs are due to dysfunction of an enzyme leading to the accumulation of substrates of this enzyme and low concentrations of its products or deficiency of a transporter which results in the accumulation of substances in one cell compartment (Fig. 1). Compromised energy production is a third group of IEMs, often referred to as mitochondrial disorders.

Many of the enzyme deficiencies lead to acute clinical symptoms via accumulation of toxic substances ('intoxication-type'-IEM) (Saudubray, Sedel, & Walter, 2006; Illsinger, 2010). The first days of life are critical for neonates with 'intoxication-type'-IEM, as every child is in a catabolic state during the post-natal period and loses weight: endogenous reserves (e.g. fat, glycogen, muscle) are used and fatty acids, amino acids and carbohydrates are released into the circulation in an uncontrolled manner. While this is unproblematic in healthy neonates it poses a critical problem in neonates affected by an IEM (Fig. 2). One of the substrates cannot be completely metabolized leading to the accumulation of toxic intermediates which finally translates into clinical symptoms (autointoxication).

Some enzyme deficiencies lead to storage phenomena where it takes time until enough storage material accumulates to cause clinical

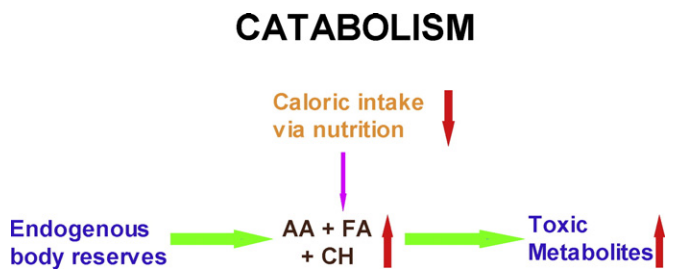

Fig. 2. Catabolism; catabolism (energy demand > energy supply) leads to an uncontrolled release of metabolites like amino acids (AA), fatty acids (FA) and carbohydrates (CH) from endogenous stores. In IEM catabolism is incomplete which results in the accumulation of toxic metabolites leading to autointoxication of the patient.

symptoms. IEMs due to transporter deficiencies usually are chronic diseases manifesting clinically in infancy, adolescence or even adulthood as it takes time until enough storage material accumulates in the respective cell compartment. Often it is not clear how storage phenomena translate into clinical symptoms. In a lysosomal storage disease, M. Anderson–Fabry, we have shown in cultured human fibroblasts in vitro that secondary disturbance of oxidative phosphorylation occurs (Das & Naim, 2009; Lücke, Höppner, Schmidt, Illsinger, & Das, 2004). This observation was later confirmed in vivo in hearts from patients with M. Anderson–Fabry (Machann et al., 2011; Palecek et al., 2010) using the MRI-technique.

Enzyme activity can be completely absent which results in clinically severe phenotypes, often there is residual enzyme activity with a clinically attenuated course of the disease.

Clinical symptoms of IEMs can be variable only if one single organ may be affected. In the 'intoxication-type' IEM frequently several organs are affected as toxic intermediates are distributed within the body via the circulation system. As the function of every cell relies on energy, disorders of energy metabolism (mitochondriopathies) are often multisystemic diseases, however due to cellular heteroplasmy only single organs may be dysfunctional as well.

3. Diagnosis of inborn errors of metabolism

At the biochemical level, IEMs due to enzyme deficiencies can be diagnosed by elevated concentrations of metabolites proximal to the deficient enzyme in the respective metabolic pathway. Metabolites distal to the compromised enzyme are low, in some diseases the ratio of metabolites proximal and distal to the enzyme is used as a diagnostic parameter (e.g. the phenylalanine/tyrosine ratio in phenylketonuria). Based on the biochemical nature of the accumulating substance different body fluids may be used like amino acids in plasma or dried blood, acylcarnitines in dried blood, organic acids in urine, amino acids in urine, mucopolysaccharides/oligosaccharides in urine, amino acids and neurotransmitters in cerebrospinal fluid.

Enzyme activities can be determined as a (confirmatory) test.

More and more genetic testing is done for the diagnosis of IEM.

In many countries a newborn screening programme for IEMs is operational. Diagnosis is based on elevated concentrations of metabolites and/or abnormal ratios of metabolites in dried blood spots (so-called 'Guthrie cards') (cf. Fig. 3). In some IEMs the enzyme activity of an affected enzyme is measured directly (e.g. in biotinidase deficiency). The target diseases included in newborn screening programmes vary considerably from country to country (Bonham, 2013). To include a pathological condition as a target disease in a newborn screening programme the criteria of Wilson and Jungner (1968) have to be met. For practical reasons the UK screening criteria are often used (Downing & Pollitt, 2008; Pollitt, 2009)

a) The natural course of the disease has to be known.
b) The condition included has to be amenable to therapy.
c) There should be a good screening test available (high specificity and high sensitivity).
d) The test should be acceptable to the public (not to much of a burden to the patient, acceptable costs for the health system, positive health technology assessment).

Ideally, a guideline should exist for any of the target diseases. It is important to be aware of the possibility of mild cases requiring (temporary) observation but no treatment ('non-disease'). The ultimate aim is to detect patients with any of the target diseases before they get symptomatic. As toxic intermediates are metabolized in utero by the mother after passing the placenta, newborn screening cannot be performed right after birth usually it is done at age 2–3 days. Untreated patients with an 'intoxication-type' IEM usually become symptomatic at days 3–6 of life thus they can be treated in the pre-symptomatic or early

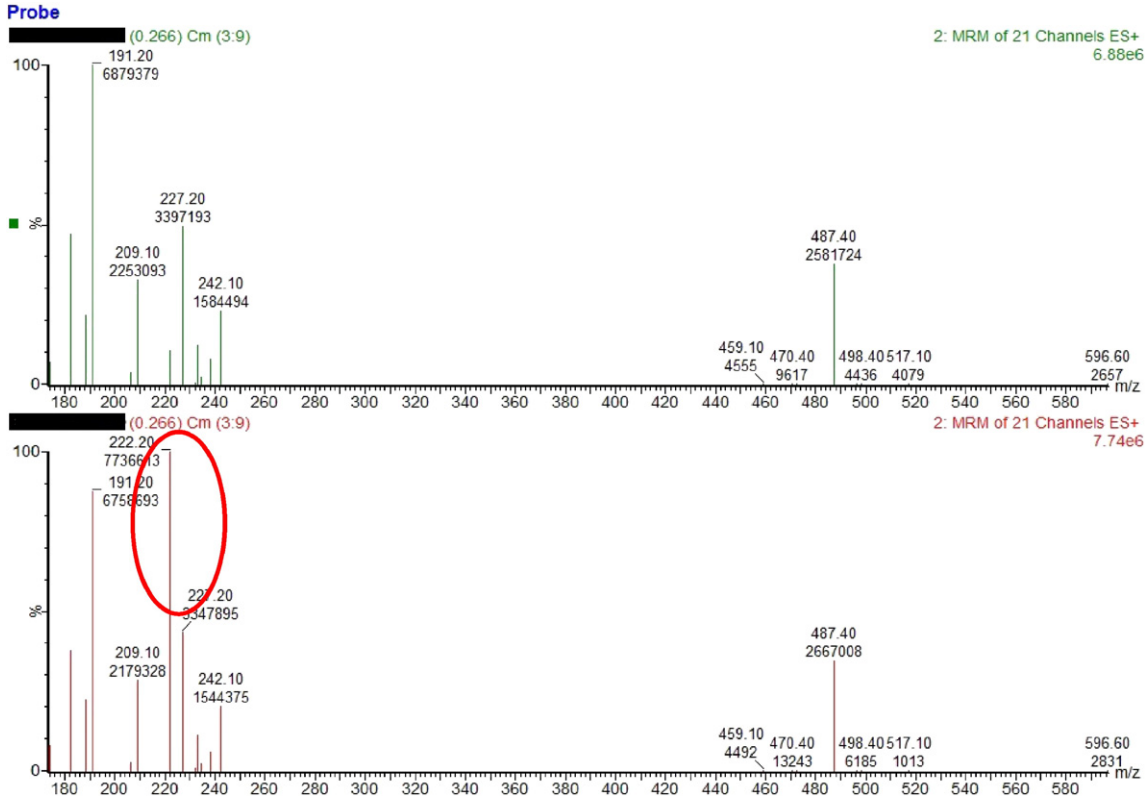

Fig. 3. Amino acid profile showing abnormal peak for phenylalanine (lower panel) at *m/z* 222. Upper panel shows a normal profile from a healthy newborn.

symptomatic stage of the disease if the newborn screening test is positive.

Not all IEMs meet these criteria and hence not all conditions can be included in a newborn screening programme. In these other diseases the IEM has to be suspected based on clinical symptoms or family history and 'selective screening' will be performed to confirm or discard the suspected diagnosis. Clinical awareness of the treating paediatrician/general practitioner for IEMs is essential.

4. Therapeutic options in inborn errors of metabolism

Based on the nature of the biochemical defect different therapeutic options can be used in IEM:

a) Substrate reduction by:

- ➢ Diet (exogenous substrates)
- ➢ Drugs (endogenous substrates)

b) Vitamins/cofactors
c) Enzyme replacement
d) Enzyme augmentation
e) Transplantation

- ➢ Organs
- ➢ Cells.

For drug development an adequate knowledge and understanding of the natural course of the disease is necessary to assess the benefit of newly developed drugs. Furthermore, the molecular pathophysiology of the disease and the mechanism of action of the proposed drug have to be known. Pre-clinical assessment by safety pharmacologists is essential to assess the risk for side effects before the drug can be tested in humans. Some guidance for drug development in the heterogeneous field of rare diseases was recently published (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidance/UCM458485.pdf).

If toxic substances/metabolites accumulate which are derived from exogenous sources like food, a diet is successful. The prototype for this therapeutic option is the treatment in classical phenylketonuria (PKU). This autosomal-recessive disease is caused by compromised function of the hepatic enzyme phenylalanine hydroxylase leading to the accumulation of its substrate phenylalanine while its product tyrosine is low. A low-protein diet (hence low in phenylalanine) is prescribed, patients are not allowed to eat meat or fish. To avoid nutritional deficiencies such a diet has to be supplemented with a special amino acid mixture free of phenylalanine containing tyrosine, essential amino acids, trace elements and vitamins. Enough calories have to be taken in order to avoid catabolism leading to uncontrolled release of phenylalanine. Outcome is generally good if treatment starts early and parents and patients comply with the dietary regimen. In the untreated patient, severe mental retardation, abnormal behaviour and epilepsy classically occur.

If toxic substances are produced endogenously pharmaceutical substrate reduction is promising like in urea cycle defects where ammonia is produced from proteolysis or in hepatorenal tyrosinaemia where toxic substances are derived from catabolism of tyrosine.

Vitamins are often cofactors of enzymes. Vitamin deficiency can then lead to dysfunction of the (intact) enzyme. Examples for this kind of disease are biotinidase deficiency or vitamin-B12 responsiveness in methylmalonic aciduria.

Enzyme replacement therapy is a quickly growing field in the last 15 years but only feasible in a small number of lysosomal storage diseases. The enzyme is produced in vitro in a bioreactor by human fibroblasts or Chinese hamster ovary cells, purified and then given to the patient intravenously. To secure uptake and lysosomal targeting of the exogenous enzyme the enzyme has to be coupled to mannose 6-phosphate which then binds to mannose 6-phosphate receptors on the cell surface thus delivering the enzyme to the lysosomal cell

compartment via the physiological sorting/trafficking process. The brain is not accessible by enzyme replacement therapy as the relatively large enzymes cannot cross the blood–brain barrier.

An allergic reaction to the enzyme infused to the patients is the most common and often very severe side effect in enzyme replacement therapy due to antibody production. The risk for allergic reactions has to be assessed by safety pharmacologists both pre-clinically and clinically. In most cases, the antibodies are not neutralizing, therefore the therapy is effective. The risk for allergic reactions and the severity differ in different diseases. While it is low in M. Gaucher and M. Fabry it is very high in infantile M. Pompe (cross-reactive immunologic material-negative) where neutralizing antibodies are formed which hamper the success of enzyme replacement therapy. The use of immunoglobulins, rituximab and methotrexate has been advocated in these patients (Messinger et al., 2012). Sometimes the full risk of side effects only becomes evident in post-marketing studies with a larger patient cohort. In principle, genotoxicity, carcinogenity and reproductive toxicity of orphan drugs have to be tested pre-clinically, however this is often not requested by the regulatory bodies, e.g. the committee for orphan medicinal products of EMA for enzyme replacement products (Joppi, Bertele, & Garattini, 2013).

If residual enzyme activity is present, enzyme augmentation is possible in some diseases using chaperones. Chaperones can (partially) correct misfolding of compromised enzymes thus improving function of the deficient pathway (e.g. Pey et al., 2008; Staudigl et al., 2011).

Organ or cell transplantation is another method to replace the deficient enzyme. Depending on the (predominant) localization of the deficient enzyme liver, kidney or bone marrow are transplanted. In severe forms of urea cycle defects or in some organic acidurias liver transplantation is indicated and leads to metabolic stabilization. To prevent rejection of the transplanted organ immunosuppression is performed. This often has side effects and patients are prone to infection and have an increased risk of post-transplant lymphoproliferative disease (PTLD). Brain function may as well be compromised due to immunosuppressive drugs. Organ transplantation is not a cure but means trading in a metabolic disease for another medical condition. Hepatocyte transplantation is an experimental method, as the cells are rejected after a few months despite immunosuppression it may be used for bridging critical conditions in very small or metabolically instable infants. Bone marrow transplantation is indicated in young children with some forms of lysosomal storage disease. It is an established therapy in mucopolysaccharidosis type I (M. Hurler) if performed in the early stage of disease before severe brain damage occurs. In contrast to enzyme replacement therapy bone marrow transplantation has an effect on the brain.

5. Pharmacotherapy in inborn errors of metabolism

In contrast to more common diseases it is a big challenge to perform studies in orphan diseases due to recruitment issues. Many centres have to be included to reach a sufficient number of patients and controlled studies are often not feasible. In many cases the full spectrum of side effects only evolves after marketing approval, often post-marketing studies are suggested or demanded by the regulatory bodies.

Pre-clinically studies by safety pharmacologists are required assessing genotoxicity, carcinogenesis and reproductive toxicity and other side effects. In OD, rarely a full set of pharmacological and toxicological studies is performed or requested by the regulatory bodies (Joppi et al., 2013). Exceptions are drugs that have originally been developed for more common diseases and were later on repurposed for OD. An example is miglustat which was originally developed for HIV-treatment and then repurposed for substrate reduction therapy in M. Gaucher and M. Niemann–Pick Type C.

In this chapter I shall describe a few examples of pharmacological therapy in inborn errors of metabolism.

Hepatorenal tyrosinaemia (HT1) is an excellent example of successful pharmacological substrate reduction. This autosomal-recessive disease is due to fumarylacetoactease-deficiency in the catabolic pathway of tyrosine metabolism (Fig. 4). Not only tyrosine is (inconstantly) elevated, succinylacetone is elevated as well as a surrogate marker for toxicity. If untreated severe liver and kidney dysfunction are observed, patients have a high risk to develop hepatocellular carcinoma (HCC). Before the advent of the substrate-reducing drug nitisinone (NTBC, 2-(2-nitro-4-trifluoromethylbenzoyl) 1,3-cyclohexanedione) HT1 was fatal, nitisinone has revolutionized the outcome in HT1. The compound was originally developed as a weed-killer; during toxicity testing in

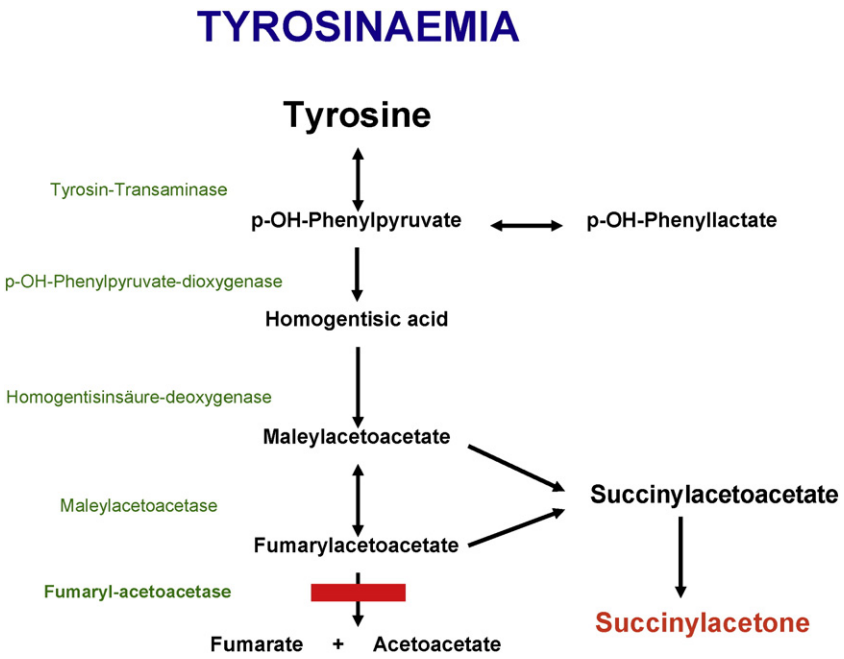

Fig. 4. Catabolic pathway of tyrosine metabolism. In HT1 fumarylacetoacetate is deficient which leads to the accumulation of both tyrosine and succinylacetone (surrogate marker of toxicity). Nitisinone (NTBC) inhibits p-OH-phenylpyruvate-dioxygenase thus suppressing the synthesis of toxic compounds.

animals it was noticed that tyrosine concentrations in blood were elevated. Further work-up showed that the enzyme fumarylacetoacetase was inhibited by this compound (Lock, Ranganath, & Timmis, 2014). This prompted colleagues in Sweden to try this substance in patients with HT1 in a compassionate use approach. The supplier of nitisinone, Zeneca, was reluctant to invest in a compound for human use with such a small target group of patients. Finally, Zeneca was persuaded to supply nitisinone for patients with HT1. First trials started in 1991 and were successful (Lindstedt, Holme, Lock, Hjalmarson, & Strandwik, 1992), the drug was used by many colleagues world-wide under a compassionate use programme (Holme & Lindstedt, 1998). In 2005 the US Food & Drug Administration and in 2005 EMA granted marketing authorization under the name of Orfadin®. Our recent multicentre cross-sectional survey has shown that early diagnosis and treatment are essential for a good outcome (Mayorandan et al., 2014). This drug leads to complete suppression of succinylacetone as a surrogate marker for toxicity. When marketing authorization was granted under special circumstances no formal studies were performed. EMA asked for a post-marketing study which did not reveal any major side effects and showed a positive effect on outcome. No pharmacological or pharmacokinetic field study results in HT1-patients are available therefore the dosing interval as well as the therapeutic concentration of nitisinone is still subject to discussion (cf. Mayorandan et al., 2014).

In urea cycle defects (UCD) substrate reduction is achieved using scavengers. The toxic compound accumulating in this group of IEMs is ammonia (Fig. 5). Hyperammonaemia leads to encephalopathy with epilepsy, brain damage/atrophy, severe psychomotor retardation and even death. Phenylbutyrate (Ammonaps®) and benzoate bind amino acids (glutamine and glycine, respectively), and water-soluble compounds are generated (Fig. 6) which can be excreted via urine. Thus, the body can get rid of nitrogen atoms without having to rely on the (defective) urea cycle. Again, data on pharmacokinetics and dynamics are scarce.

Enzyme replacement therapy (ERT) is a rapidly growing field offering therapy in a group of IEMs called lysosomal storage diseases. Table 1 summarizes the currently available therapeutic preparations for lysosomal storage diseases in the European Union. The high prices reflect the burden and challenge of developing drugs for only a few patients, recruiting patients with an OD for studies and the sophisticated production process using cell cultures. Health technology assessment has shown good cost-effectiveness of ERT in Gaucher Type 1 disease (Van Dussen, Biegstraaten, Hollak, & Dijkgraaf, 2014) but it was lower in M. Fabry (Rombach, Hollak, Linthorst, & Dijkgraaf, 2013) and M. Pompe (Kanters et al., 2014). Clinical symptoms improve in most patients,

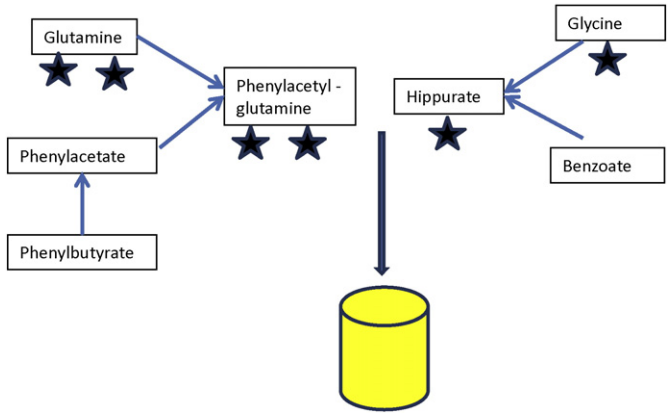

Fig. 6. Nitrogen scavengers in UCD. Phenylacetate is conjugated with glutamine producing water-soluble phenylacetyl-glutamine. Benzoate is conjugated with glycine forming water-soluble hippurate. Asterisks represent nitrogen atoms.

rarely patients get completely free of symptoms. Allergic reactions are common in ERT, however the safety profile of ERT in M. Fabry is judged to be positive (Barbey & Livio, 2006). Again, post-marketing studies including the Fabry Outcome Survey (FOS) were necessary to assess safety and efficacy in a bigger cohort of patients. The major drawback of ERT is a lack of effect on cerebrospinal symptoms as the enzymes are unable to cross the blood–brain barrier.

Tetrahydrobiopterin (BH4) is an essential cofactor of phenylalanine hydroxylase. IEMs of BH4-synthesis or BH4-recycling result in low concentrations of BH4, hence dysfunction not only of phenylalanine hydroxylase but of tryptophane- and tyrosine hydroxylase in the brain as well. This results in elevated phenylalanine concentrations and reduced levels of the neurotransmitters serotonin and dopamine. This disease is called 'atypical' phenylketonuria. BH4 is not able to cross the blood–brain barrier, hence it does not have an effect on defective neurotransmitter synthesis, however phenylalanine concentrations are normalized. Neurotransmitter precursors have to be supplemented. For many years, BH4 was used as a chemical compound to treat patients with 'atypical' PKU. Since a few years, Kuvan® (BH4) is licenced in the European Union. The safety profile as well as efficacy is good.

Kuvan® can also be used as a chaperon in some patients with clinically milder mutations (Pey et al., 2008). It improves protein folding and increases enzyme activity thus allowing a higher protein tolerance.

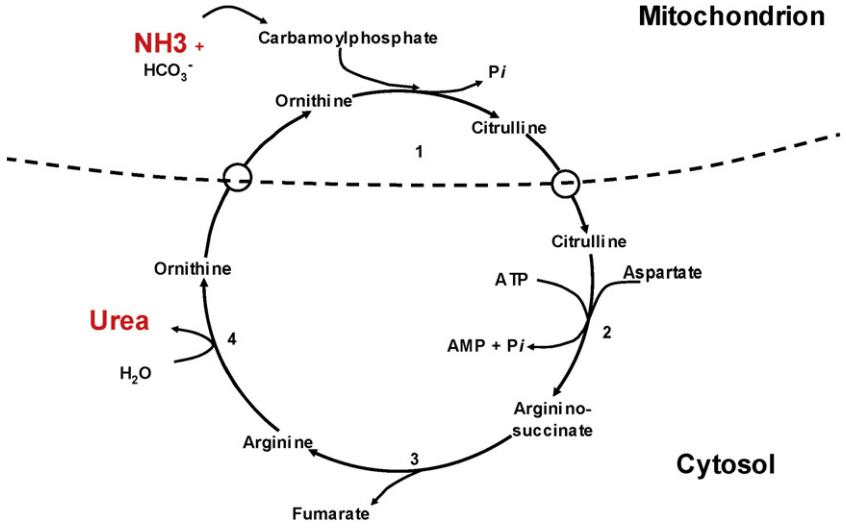

Fig. 5. Urea cycle. Ammonia (containing nitrogen) is metabolized to water-soluble urea which can be excreted via urine.

Table 1
ERT available for lysosomal storage diseases in the European Union. Costs refer to an adult of 70 kg (retail prices in Germany for the year 2016).

Disease	Preparation for ERT	Prevalence	Annual costs
M. Anderson–Fabry	Fabrazyme	0.22:100,000	255,093 €
	Replagal		256,255 €
M. Gaucher Type I	Cerezyme	1:100,000	652,513 €
	VIPRIV		635,095 €
M. Hunter (MPS II)	Elaprase		1,085,111 €
M. Hurler–Scheie (MPS I)	Aldurazyme	1:100,000	662,388 €
M. Maroteaux–Lamy (MPS VI)	Naglazyme		1,371,275 €
M. Morquio (MPS IV)	Vimizyme		1,645,000 €
M. Pompe (GSD II)	Myozyme	0.3–7:100,000	491,065 €

6. Summary

Diagnosis and treatment in patients with OD is difficult and challenging, it often takes many years of a diagnostic odyssey until a diagnosis is established (Dudding-Byth, 2015).

For some OD good therapeutic options exist. Safety and efficacy of drugs have to be demonstrated in pre-clinical and clinical studies before marketing authorization is granted, often post-marketing surveys are however necessary to gain more comprehensive data.

IEMs are taken as an example for OD. Dietary intervention is successful in some IEMs, while in others pharmaceutical intervention is necessary to improve the outcome. For a few patients ERT is available. Some patients that cannot be stabilized by dietary and pharmacological therapy have to undergo organ or cell transplantation.

Conflict of interest statement

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