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Minireview

Taliglucerase alfa: An enzyme replacement therapy using plant cell expression technology

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ABSTRACT

Gaucher disease (GD) is a rare, genetic lysosomal storage disorder caused by functional defects of acid β-glucosidase that results in multiple organ dysfunction. Glycosylation of recombinant acid human β-glucosidase and exposure of terminal mannose residues are critical to the success of enzyme replacement therapy (ERT) for the treatment of visceral and hematologic manifestations in GD. Three commercially available ERT products for treatment of GD type 1 (GD1) include imiglucerase, velaglucerase alfa, and taliglucerase alfa. Imiglucerase and velaglucerase alfa are produced in different mammalian cell systems and require production glycosylation modifications to expose terminal α-mannose residues, which are needed for mannose receptor-mediated uptake by target macrophages. Such modifications add to production costs. Taliglucerase alfa is a plant cell-expressed acid β-glucosidase approved in the United States and other countries for ERT in adults with GD1. A plant-based expression system, using carrot root cell cultures, was developed for production of taliglucerase alfa and does not require additional processing for postproduction glycosidic modifications. Clinical trials have demonstrated that taliglucerase alfa is efficacious, with a well-established safety profile in adult, ERT-naïve patients with symptomatic GD1, and for such patients previously treated with imiglucerase. These included significant improvements in organomegaly and hematologic parameters as early as 6 months, and maintenance of achieved therapeutic values in previously treated patients. Ongoing clinical trials will further characterize the long-term efficacy and safety of taliglucerase alfa in more diverse patient populations, and may help to guide clinical decisions for achieving optimal outcomes for patients with GD1.

Contents

1. Introduction

Gaucher disease (GD) is a rare lysosomal storage disorder that results from mutations in the gene (*GBA1*) encoding acid β-glucosidase (glucocerebrosidase [EC 3.2.1.45]) [1]. Insufficient enzyme activity leads to accumulation of glucosylceramide and other glucosphingolipids within the lysosomes of various cells and tissues, which results in varying degrees of visceral, bone, and neuronal pathology [1]. Three types of GD have been identified; GD type 1 (GD1) is the most common in the Western world, with clinical manifestations of hepatosplenomegaly, anemia, thrombocytopenia, and bone and bone marrow disease, but a lack of early onset primary central nervous system involvement. The

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latter is characteristic of GD types 2 and 3. Currently, enzyme replacement therapy (ERT) with repeated intravenous infusions of recombinant, active acid β-glucosidase ameliorates many of the visceral signs and symptoms, and is recommended for patients significantly affected by GD1 [1,2].

Early attempts to treat GD1 with intravenous ERT were unsuccessful and provided limited therapeutic benefit, primarily because: 1) exogenous administration of unmodified human enzyme derived from placenta did not effectively enter target cells [3]; and 2) the amounts of such enzyme preparations were severely limited. Modern ERT evolved based on the ability to produce large amounts of human enzymes by recombinant methods and discovery of the macrophage mannose receptors (MMRs) for preferential uptake of mannose-terminated glycoproteins including acid β-glucosidase, by tissue macrophages [4,5]. This finding spurred development of more effective recombinant human acid β-glucosidase products, modified using various posttranslational or postproduction glycosylation methods to expose terminal α-mannose residues [6,7]. Currently, three ERTs are commercially available for the treatment of GD1; two are produced in mammalian cell-based systems. Imiglucerase (Genzyme, a Sanofi Corporation, Cambridge, MA, USA) is produced in a Chinese hamster ovary [CHO] system and velaglucerase alfa (Shire Pharmaceuticals Inc., Dublin, Ireland) is produced in a human fibrosarcoma cell line [8–10]. Taliglucerase alfa (Protalix Biotherapeutics, Carmiel, Israel) is a new acid β-glucosidase ERT that is approved by the US Food and Drug Administration for the treatment of GD1 in adults in the United States [11]; it is also approved in Israel and other countries, and is the first plant cell-expressed biotherapeutic approved for use in humans [12].

While the use of mammalian cell-based systems for the production of biotherapeutics is well established [13], manipulation of plant-based platforms for production of biotherapeutic agents is relatively new [14]. In recent years, researchers have successfully produced recombinant human proteins from whole-plant, transgenic crops [15]. In addition to transgenic plants, other production platforms are available, such as those based on plant-transient expression technology (virus-based or not), which are being used for vaccine antigen production [16]. Taliglucerase alfa is unique in that it is a plant cell-expressed biotherapeutic produced in a closed, sterile culture system [12]. Because of the novelty of the plant cell-based protein production system, a key purpose of this review is to describe the production technology for taliglucerase alfa. Furthermore, the clinical implications of this enzyme expression system will be discussed in the context of completed and ongoing clinical studies of this novel ERT.

2. Expression and production platforms for biotherapeutics

As the development and manipulation of expression platforms for biotherapeutics have progressed, distinct advantages and disadvantages have emerged with each approach (Table 1) [14,17–19]. Advantages of prokaryotic production systems (e.g., *Escherichia coli*) include ease of genetic manipulation, rapid cell growth, and high levels of protein expression [13]. However, prokaryotic systems are not ideal for the production of complex proteins that require posttranslational modifications for therapeutic efficacy. Posttranslational modifications of protein folding or disulfide bond formation are difficult to achieve with prokaryotic systems, and posttranslational glycosylation processing is not possible.

Since yeast- and mammalian cell-based production systems are capable of similar posttranslational modifications, they provide for the production of therapeutically active proteins compared with prokaryotic platforms [13,20]. These systems can be more expensive than prokaryotic systems, specifically in terms of culture processes, operating costs, and scale-up difficulties [18]. Furthermore, compared with prokaryotic systems, these systems typically produce lower yields [13,18,19].

Plant-based production systems share some advantages and disadvantages of both prokaryotic and mammalian systems. Like prokaryotic systems, plant cell-based systems are associated with low culture costs, as they permit use of simple synthetic media, and allow rapid scale-up of the enzyme product [14,17–19]. In addition, plant-based systems are capable of expressing complex proteins, and effectively achieve posttranslational modifications, specifically *N*-glycosylation, as with other widely used yeast and mammalian systems [13,14,21]. Because plant cell-based systems do not use cells or culture media from animal sources, expression products are not susceptible to contamination with mammalian pathogens [14]. Moreover, plant cell cultures present a natural barrier to mammalian pathogen contamination, as attempts to propagate mammalian viruses in plant cells have been unsuccessful [22]. Nevertheless, plant-based systems present distinct challenges, depending on the specific plant system utilized [14,19].

2.1. Taliglucerase alfa plant-based production platform

Taliglucerase alfa is produced using the novel ProCellEx® plant cell-based protein expression system [12], in which plant cells are cultured in suspension in a closed bioreactor (Fig. 1). Isolated carrot root cells were stably transformed with *Agrobacterium tumefaciens* carrying the binary Ti-plasmid vector harboring the acid β-glucosidase cDNA and the kanamycin resistance gene (*NPTII*). Transformed cells were selected by antibiotic resistance, and clonal selection was performed by analysis of protein expression levels in transgenic calli grown on solid medium [14,23]. The selected clone was further expanded in suspension to develop a master cell bank consisting of frozen cell aliquots similar to the mammalian systems, in order to provide a continuous supply of fresh cells for inoculation into bioreactors [24].

For cell culture, the thawed cells were initially seeded in solid media, and the resulting cell mass was used to inoculate the liquid medium.

Table 1
Overview of the advantages and disadvantages of protein expression systems.

Advantages	Disadvantages
Prokaryotic systems [14,17,18]	
• Ease of genetic manipulation	• Difficulty with posttranslational modifications (signal peptide cleavage, propeptide processing, protein folding, disulfide bond formation, and glycosylation)
• Rapid growth	
• High expression level	
Mammalian-based systems [14,17–19]	
• Improved posttranslational modification	• High costs
• Production of therapeutically active proteins	• Low yields
	• Scale-up difficulties
Plant-based systems [14,17]	
• Lower costs	• Protein instability/degradation
• Easy transformation	
• Improved posttranslational modification	
• Decreased risk of contamination with mammalian pathogens	
• Efficient, readily scaled up	