Supply, pharmaceutical qualification and biopharmaceutical consideration of amyloglucosidase solution in congenital and acquired sucrase-isomaltase deficiency treatment

Rémi Lambert[1,2], Alain Lachaux[3], Agathe Louguet[1,2], Constantin Dupuis[1,2], Benjamine Lapras[1,2], Carole Paillet[2], Christine Pivot[2], Samira Filali[1,2], **Camille Merienne[1,2]**, Fabrice Pirot[1,2]

[1] Unité de Préparation et de Contrôle des Médicaments, plateforme FRIPHARM. [2] Pharmacie à usage intérieur, Groupement Hospitalier Edouard Herriot - Hospices Civils de Lyon. [3] Service de gastroentérologie, hépatologie et nutrition pédiatrique, Hôpital Femme Mère Enfant - Hospices Civils de Lyon et Université Claude Bernard Lyon 1, Lyon, France.

COM21-69138

OBJECTIVES

To propose a pharmaceutical qualification (PQ) of a food grade amyloglucosidase (AMG) solution and to evaluate its risks and specifications as an enzyme replacement therapy in sucrase-isomaltase deficiency (SID) treatment

BACKGROUND

Congenital SID is a rare genetic condition, which appears during food diversification. An acquired deficit is also possible in association with various digestive diseases. It affects the ability to hydrolyze sucrose and starch into monosaccharides. Primary or secondary deficiencies are responsible of a wide variety of phenotypes that reveal after ingestion of sucrose or starch, by stomach cramps, bloating, diarrhea and vomiting. These digestive problems can lead to failure to thrive and malnutrition.

Enzyme replacement therapy offers an alternative to sucrose and starch free diets to treat symptoms in SID. Our pharmaceutical platform already proposes an invertase solution (11 600 IU/mL) to improve sucrose digestion.

We want to propose an AMG solution as supplement to invertase solution for completing SID treatment. AMG is an enzyme derived from *Aspergillus niger* strains, widely used in brewing and alcohol distillation for it ability to hydrolyse starch and maltose to glucose. However, taking into account its origin, a PQ is required.

MATERIAL AND METHODS

In accordance with the European Pharmacopoeia concerning substances for pharmaceutical use, several tests were performed:

➢Conformance of physicochemical characteristics such as organoleptic characteristics, density (calculated by weighing a precise volume of solution), pH, osmolality (measured by freezing point depression)

➢Determination of the enzymatic activity (AE) of AMG by a colorimetric enzymatic method using a reagent containing p-nitrophenyl-β-D-maltoside plus a thermostable β-glucosidase enzyme in excess (Fig. 1). The AE of AMG was measured under two experimental conditions:

(i) at 40 ° C and pH = 4.5, in accordance with the recommendations for the use of the reagent
(ii) at 37°C and pH = 6, to evaluate the enzyme activity expected in physiological conditions of the duodenum [1] and to define the optimum dose.

➢Control of the microbiological quality of the solution in accordance with the EP concerning the microbiological quality of non-sterile substances for pharmaceutical use. A bioburden determination was carried out to verify:

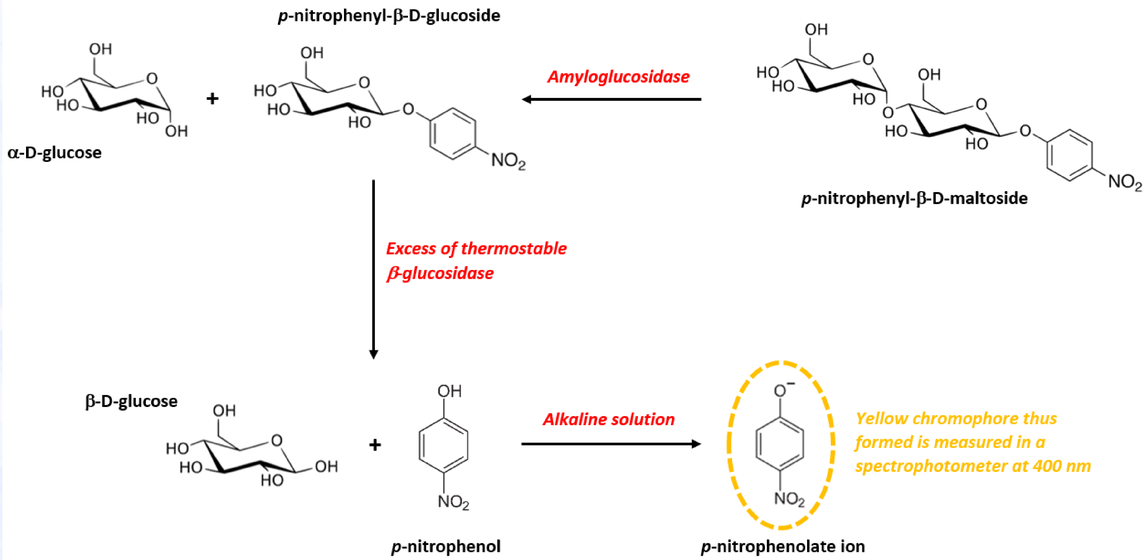
- TAMC (total aerobic microbial count) < 10^2 CFU/mL
- TYMC (total combined yeasts/moulds count) < 10 CFU/mL
- Absence of *Escherichia coli* in 1 mL

RESULTS

The results obtained are shown in the table:

Parameters	Values
Density	1,13
pH	4,5 ± 0,5
Osmolality (1/10 diluted solution)	400 ± 20 mOsm/kg
EA at conditions (i)	5275 ± 400 UI/mL
EA at conditions (ii)	1320 ± 70 UI/mL
Microbiological quality	Sterile culture

Figure 1 : mécanisme d'action du dosage de l'AE de l'AMG



DISCUSSION

The organoleptic characteristics, pH and osmolality were conformed to the supplier specifications. Because of high osmolality, the AMG solution has to be diluted ten times before use.

According to the EP, the microbiological quality of the solution was conformed. For the PQ of the product, some further tests will be performed to check residual solvents.

Considering (i) the starch amount ingested per day by children depending on weight and (ii) the enzymatic activity of the AMG solution, the optimal dosage was defined as follows:

➢ 2 mL per meal for children under 15 kg
➢ 4 mL per meal for children over 15 kg

The safety of the AMG solution excipients was verified.

CONCLUSION

In order to improve SID, AMG solution will be proposed to 25 patients already treated by our invertase solution.

[1] Fallingborg J. Intraluminal pH of the human gastrointestinal tract. Dan Med Bull.1999 Jun;46(3):183-96
[2] Fantino M, Gourmet E. Apports nutritionnels en France en 2005 chez les enfants non allaités âgés de moins de 36 mois. Archives de pédiatrie. 2008;15(4):446-455

Congress 2021 : 24th GERPAC days