FOOD-CT-2005-007036

EARNEST

EARly Nutrition programming- long term follow up of Efficacy and Safety Trials and integrated epidemiological, genetic, animal, consumer and economic research

Instrument: Integrated Project

Thematic Priority 5.4.3.1: Food Quality and Safety

Final public report on activity 6.2.1.

Title of activity: Formulation of BSSL for clinical use

Period covered from 15.04.2005 to 14.10.2010

Start date of project: 15.04.2005 Duration: 5,5 Years

Organisation Name of Lead Contractor for this report: ORDESA

Formulation of BSSL for clinical use

Participating partners: Biovitrum, Ordesa

Objective

The objective of this activity was to develop a suitable formulation of the recombinant human enzyme bile-salt stimulated lipase (rhBSSL) for its administration to preterm infants during the clinical study covered by activity 6.2.2.

Development of rhBSSL

Development of a BSSL manufacturing process involved the selection of a production mammalian cell line, purification methods, methods for virus clearance and methods for analysis of the final product. All steps to produce rhBSSL were according to Good Manufacturing Practices.

Toxicology studies with rhBSSL in different animal models such as juvenile rats and marmosets were also performed, and generated data to support safety of rhBSSL. In such studies, no local or systemic toxicity was observed after doses up to 115 mg/kg/day.

BSSL was formulated in an appropriate pharmaceutical form, stable, soluble in water and easy to dose. BSSL was delivered as a frozen oral solution in a 10 mL glass vial containing 15 mg/mL BSSL.

It was established that for clinical use, BSSL solution should be added to the study preterm formula prior administration to the preterm infant.

The chemical stability of rhBSSL added to preterm infant formula (developed by Ordesa and used in the ongoing clinical trial) at a concentration of 150 mg/L was investigated. The mixtures were stored at 5 ± 3 ℃, ambient temperature and 37 ± 2 ℃ for 24 hours and the concentration of rhBSSL was measured as enzyme activity before and after storage. The concentrations of rhBSSL-DS were found to be approximately 90 and 100% of the initial concentrations after storage for 24 hours at 5 ℃ and ambient temperature, respectively. The rec overy at 37 ℃ after storage for 3 hours was 109 %. The results indicate acceptable chemical stability of rhBSSL-DS when mixed to infant formula and stored refrigerated for 24 hours, including temporary temperature excursions during handling and administration.

Preterm Formula development

A preterm formula was specially developed for this study. Nutrient composition was defined together with scientific experts and according to recommendations of International Pediatric Societies. Formula supplied 81 kcal, 2,3 g of proteins, 4,1 g of fat and 8,7 g of carbohydrates per 100 ml, and was supplemented with the long-chain polyunsaturated fatty acids Arachidonic acid (0,5% of total fatty acids) and Docosahexanoic acid (0,35% of total fatty acids). Selection of ingredients and procedures were according standard practices.

Formula was delivered in a ready-to use form in 90 ml glass bottles which were filled in sterile conditions. Pilot production of the formula was performed and stability tests throughout 6 months completed the product development phase.

Supply of rhBSSL and preterm formula

BSSL and preterm formula were manufactured in several batches to cover all the clinical study period. They were supplied by Biovitrum and Ordesa directly to the following centres participating in the clinical trial, according to their needs:

- *Polytechnic University of Marche and Salesi Children's Hospital, University of Ancona, Ancona, Italy (Principal Investigator: Professor Carnielli)*
- *Policlinico 'Agostino Gemelli' Catholic University of the Sacred Heart, Rome, Italy*
- *Azienda Ospedaliera di Padova-Dipartimento di Pediatria Padua, Italy*
- *U.O. Neonatologia, Patologia Neonatale e Terapia Intensiva – Azienda Universitaria Policlinico Umberto Rome, Italy*
- *Azienda Ospedaliera "Ospedale Policlinico Consorziale"-U.O. Neonatologia e Terapia Intensiva Neonatale Bari, Italy*

Conclusions

In conclusion, the objective of this activity was successfully achieved since it was possible to develop and find a suitable formulation of BSSL for use in preterm infants that was tested in the pilot clinical trial covered by activity 6.2.2.