Individual All-in-One Parenteral Nutrition Admixtures for the Organ Replacement of Infants

PhD thesis

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Introduction

The feeding of sick children admitted to hospital per age group and diseases is a task requiring very great patience and persistence from healthcare professionals. Particularly challenging for clinicians, to build up and maintain a unique quality and quantity of nutrition through a shorter or longer period of time for infants unable to digest food via the gastro-intestinal tract. In patients with severely impaired digestive systems the poor nutrition quickly leads to the development of malnutrition.

There is a great need for individual variability in artificial nutrition, particularly in the case patients of partial or entire colon and/or 50% small intestine removal resulting in nutrient deficiency and generalized malabsorption.A very small, 10 cm or larger small intestine is sufficient forchildren's survival, which determines the length of time of later parenteral nutrition, or even the oral nutrition ability.

The technical development of surgery, the modern intensive care after intestine removal and the organ-specific parenteral nutrition supplements allow the survival of numerous neonatal and infant with short bowel syndrome (SBS).Due to the growth capacity of the intestines in infants, enteral feeding is of better prognosis than in adults; 70-80% in 1-2 years may be switched to oral feeding.The disease mortality is decreasing, thus the importance of nutrition at home become more appreciated.The component therapy in hospital wards is of sepsis dangerous and therefore it should be prepared in pharmacy having proper human and infrastructural conditions.In our country, the role of pharmacists in nutrition therapy is more self-motivated, but we can ensure proper feeding of patients with a moral imperative and as a part of the healing effectiveness.

In my dissertation I presented a new magisterial all-in-one parenteral nutrition (Magi-AIO-TPN) admixtures planning, which I worked out based on literature data. For me it was especially important that the children’s clinical nutrition therapy should be carried out more easily and safely. Therefore my primary purpose was to assure a commensurable stability of the individually tailored Magi-AIO-TPN admixtures with the similar three chamber pre-filled commercial bags mixed before the application by breaking the seals between the chambers by rolling and then adding the necessary trace elements, vitamins and other ionic compounds according to the patients’ needs.

My other purpose was to improve the children’squality of life, to become possible the home care parenteral nutrition therapy.

Aims

The purposes of my thesis were the followings:

• To develop parenteral nutrition plan tables for children, and their adaptation into the pediatric practice,

• To provide the opportunity for home care total parenteral nutrition therapy with magistral all-in-one parenteral nutrition admixtures (Magi-AIO-TPN),

• The define the optimum storage conditions of Magi-AIO-TPN admixtures on the basis of the results of microbiological and physical-chemical stability examinations,

•To track the effect of Ca and Mg in the forms of their inorganic and organic acid salts on the stability of (Magi-AOI-TPN) admixtures,

•To track the degradation kinetics of sensitive components (eg. Vitamin C, glutamine) in the Magi-AIO-TPNs.

Methods

Planning of the composition of Magi-AIO-TPN admixtures

For the individually tailored preparation of Magi-AIO-TPN admixtures, I worked out an auxiliary table to plan the compilation. The first step was to conversion of the g/kg/day, mmol/l/kg/day data to ml/kg/day dimension. By using the auxiliary table the children's individual all-in-one parenteral nutrition needs can be calculated directly considering their age, body weight (maxium-minimum values), their recent laboratory results and current status. In order to determine the optimum storage conditions of the Magi-AIO-TPN admixtures, each sample was stored at 2-8 ºC, 25°C and 30°C for 14 days, in the case of mean droplet size measurements the storage time was 28 days.

For the model Magi-AOI-TPN admixture a nutrient composition of a three year old, satisfactory weight (25 percentile, -1SD) and 25% under-calorized child was chosen with relatively high ion (Na^+, K^+, Ca^{2+}) concentrations. My goal was to examine the stability of anextreme nutrient composition.

I examined and compared the microbial and physico-chemical stability of Magi-AIO-TPN admixtures containing various organic and inorganic Ca and Mg compounds with and without Soluvitas a function of storage.

Microbiological examinations

The parenteral formula was studied at three different temperatures (2-8ºC, 25ºC, 30ºC) for 14 consecutive days long. Aerobic bacterial and mycological cultures were performed according to the pharmacopoeia monograph.

The samples were treated and evaluated according to the rules of microbiological sample processes. 10 μl of test TPN samples were inoculated onto Columbia agar containing 5% sheep blood, onto chocolate agar containing polyviteX and onto fungal Sabouraud medium (BioMérieux plates). They were incubated at 37°C for 24 hours, than stored at room temperature for another 24 hours. Reading and evaluation of the discs were carried out after 24 and 48 hours. 1 ml of the TPN solution was inoculated onto medium, which contains hemin and vitamin K3 thioglycolate, and incubated at 37°C for 24 hours. The enriched sample was processed and evaluated in the same manner of the direct blanking.

Zeta-potential measurements

Zeta potential measurements were carried out at 25°C using Zetasizer Nano ZS apparatus (Malvern Instruments, UK). An electric field was applied to the dispersion of particles, which would then move with a velocity related to their zeta potential. This velocity was measured using a patented laser interferometric technique called M3-PALS (Phase Analysis Light Scattering). This enables the calculation of electrophoretic mobility, and from this the zeta potential for the accurate measurement using the Smoluchowsky formula and expressed in mV.Measurements were carried out at 25±1°C with the freshly prepared emulsions and also, with the samples stored for 0 to 14 days on 3 different temperatures. Particle size range for zeta potential measurement is from 5 nm to 10 μm.

The evaluation of zeta potential measurements indicates the instability of these systems with the values smaller than ±30 mV. The results showed decreasing tendency especially in samples stored on low temperature.

Particle size measurements

Mean droplet size (MDS), size distribution and polydispersity of the emulsion droplets were measured at 25°C using Zetasizer Nano ZS apparatus (Malvern Instruments, UK).

Dynamic Light Scattering(DLS) is used to measure particle diameter. This technique measures the diffusion of particles moving under Brownian motion, and converts this to size and a size distribution using the Stokes-Einstein relationship. Non-Invasive Back Scatter technology (NIBS) is incorporated in the instrument to give the highest sensitivity simultaneously with the highest dynamic size and concentration range.It includes 2 angle size measurements for the enhanced detection of aggregates, measurement of small or dilute samples, or at high concentration. In addition, the optics is not in contact with the sample and hence the detection optics are said to be non-invasive. The measurement position within the cuvette of the Nano ZS is automatically set to accommodate the requirements of high sensitivity or high concentration. This position is changed by moving the focusing lens.

Particle size range for size measurement is from 0.6 nm to 6 μm.

Surfacetension measurements

The surface tension of the Magi-AOI-TPN admixtures was determined by a dynamic surface tension method using Du-Noüy ring and Wilhelmy plate with a computer-aided dynamic tensiometer(KSV Sigma 70, RBM-R. Braumann GmbH, Germany).

Statistical Evaluation

Average particle size and zeta-potential values of mixtures at different temperatures and storage intervals were compared using the two-sample t-test assuming equal variances. In this case, the comparison was made between infusions stored at different temperatures.The statistics were calculated using Microsoft Excel 2003.

Determination of the ascorbic acid concentration

The measurement was the internal standard method with 3-hydroxy-butyric acid internal standard. The ascorbic acid was diluted in deionised water to obtain solutions at appropriate concentrations for implementation. They were freshly prepared before use. The samples were ultrafiltrated with 10 kDa filter (Millipore) at 14000 rpm before measuring them. The ESI-MS/MS was API 4000 QTRAP MS/MS mass spectrometer equipped with Perkin Elmer 200 LC. The ESI-MS/MS system was used in negative ion mode, the quantification of the ascorbic acid ($M(M-H^+)$=175) and the internal standard ($M(M-H^+)$=103) based on transitions of *m/z* 175→115, *m/z* 175→87 and *m/z* 103→77. For the optimum MS/MS performance the measuring parameters were the followings: ion spray voltage: -4500 V, ion source temperature: 200 °C, declustering potential: -60 V, entrance potential: -10 V, collision energy -24 V. 20 μl sample was injected in infusion mode. The eluent was water:acetonitrile mixture (80:20) with flow rate of 200 μl/min.

Examination of thestability of L-alanyl-L-glutamine

The stability assay took 14 days at three temperatures (2-8°C, 25°C, 30°C). The measurement was the internal standard method with glycylglycine internal standard. The L-alanyl-L-glutamine was diluted in deionised water to obtain solutions at appropriate concentrations for implementation. The ESI TSQ Quantum Discovery triple quadrupole mass spectrometer equipped with Jasco X-LC binary pump and Jasco X-LC autosampler. The ESI-MS/MS system was used in positive ion mode, the quantification of the L-alanyl-L-glutamine ($M(M+H^+)$=218) and the internal standard ($M(M+H^+)$=133) based on transitions of *m/z* 218→84, *m/z* 218→130, *m/z* 218→147 for, and *m/z* 133→76, *m/z* 133→87. The MS parameters were the followings: spray voltage: 4000 V, sheat gas pressure: 35 psi, aux gas pressure: 5 psi, capillary temperature: 300 °C, collision pressure: 1.0 mTorr, scan time: 0.3 s, tube lens offset: 70 V.

RESULTS

- In my thesis I presented the Hungarian implementation of Magi-AIO-TPN admixtures in pediatric use. The appropriate stability of the prepared systems were confirmed by physicochemical and microbiological examination under 3 different storage temperatures for 14 days of storage with daily sampling and completed with additional data on day 21 and day 28 measurements.
- Since 2003 based on the AIO parenteral nutrition experience of 80 infants, I worked out a preparation protocol which can be adapted to hospital pharmacy laboratory conditions.
- The results of microbiological, physical and chemical stability studies supported the acquired experience in more than a decade of correct nutrition therapy, as evidenced by the improvement in the quality of life of long parenterally fed children, as well.
- Based on the results of stability studies of Magi-AIO-TPN admixtures containing organic and inorganic Ca and Mg salts the preparation costs can be reduced. The use of admixture type B/III (Mg-aspartate - Mg sulfate exchange) could enable the extension of storage timeto 10 days at 2-8 ° C, and to 14 days at 25 ° C storage temperature, respectively, with the application of 5 μm diameter filter.
- The zeta potentialsof the homogenized samples containing extreme ion compositions reserved their physical stability (their values are around -20 mV). - The surface tension values show no significantly change for 13 days at 25 °C, and for 10 days at 2-8°C and 30 °C.
- The application of 5 μm diameter filters can increase the patient' safety in home care parenteral nutrition practice, since the results carried out for 28-day studies demonstrate that each admixture can be safely administered even in case of higher storage temperature fluctuations.
- I proved the acceptable stability, thus the miscibilityof glutamine to the amino acid infusion in the course of the preparation of admixtures, while as it was expected, I also confirmed that after 48 hour-storage no ascorbic acid remained in the solution at 2-8°C, thus it should be added to the infusion daily.

Conclusion

The work presented in my thesis shows the validity of the decade long empirical approach used in the planning and preparation of the Magi-AOI-TPN emulsions.Additionally the quantitative measurements prove that the emulsionscan be used over 10 days long period and are not sensitive to storage temperature; the results even show more uniform values and consequently better stability under room temperature storage.

Practical use

- The introduction of the auxiliary table to the daily nutrition therapy enabled the improvement of the quality of practice, by decreasing the errors associated with the nutrition therapy, by reducing nurse work and improving the quality of life of patients, leading to the implementation of the Home Parenteral Nutrition of numerous beneficial aspects. This example demonstrates how an aseptic, safe environment can be created by compounding of all-in-one parenteral nutrition admixtures under laboratory conditions instead of hospital wards.
- The results confirm the correctness of our 13 years of empirical practice, that no side effects were found with the application of nutrition admixtures of stored for 6-7 days. Cholestasis has not been observed at two long-time (for 9 and 5 years) parenterally fed patients, their liver enzymes values remained in the normal range (ALT/AST 20-40 U/L).
- With the publication of my results, I contributed to the selection of Home Parenteral Nutrition Centers, which led to the starting of the financing of parenteral nutrition home care therapy. In Hungary our Clinic is the only one where exist a Children's Home Parenteral Nutrition Center.

List of original publications

Publications connected to the Ph.D. thesis

1. **Turmezei J,** Jávorszky E, Szabó E, Dredán J, Kállai-Szabó B, Zelkó R. (2015) Effect of storage temperature on the stability of total parenteral nutrition admixtures prepared for infants. ActaPoloniae Pharm–Drug Res, 72(5) : 843-849.

2. Telessy IG, Balogh J, **Turmezei J**, Dredán J, Zelkó R. (2011) Stability assessment of o/w parenteral nutrition emulsions in the presence of high glucose and Calcium concentracions. J Pharm Biomed Anal, 56(2): 159-164.