Chapter 6

Neurodevelopmental outcomes of very low birth weight infants after enteral glutamine supplementation in the neonatal period


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Abstract

Aim: To determine the effect of neonatal glutamine-enriched enteral nutrition in very low birth weight (VLBW) infants on neurodevelopmental outcome at two years of age

Methods: Of the 102 infants of the initial study (13 died, 1 exclusion due to a chromosomal abnormality), 88 were eligible for the follow-up study. Neurodevelopmental outcome (neurologic status, vision, hearing, and Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI) of the Bayley Scales of Infant Development II was evaluated at the corrected age of two years. To adjust for potential confounders, data were analyzed by multiple linear or logistic regression for continuous and dichotomous variables, respectively.

Results: 72/88 (82%) infants participated in the follow-up study: 40 in glutamine supplemented group and 32 in control group. The incidence of either a MDI or a PDI ≤ 85 was not different in glutamine supplemented and control group (MDI ≤ 85: 27 and 19%, p=0.17; PDI ≤ 85: 28 and 16% p=0.16 respectively). The incidence of neurodevelopmental impairment was not different between both groups (OR: 2.16, 95% CI: 0.64-7.28).

Conclusions: Although neonatal glutamine-enriched enteral nutrition in VLBW infants reduced neonatal infectious morbidity, it did not improve neurodevelopmental outcome at two years of age in this study.
Introduction

Several studies in very low birth weight (VLBW) infants have investigated the effect of parenteral or enteral glutamine supplementation on morbidity and short term outcome in the neonatal period.\textsuperscript{1-5} In recent studies in VLBW infants, Neu et al.\textsuperscript{1} and our group\textsuperscript{2} have found that glutamine-enriched enteral nutrition between day 3 and 30 of life decreased the incidence of neonatal infections. Other studies on enteral\textsuperscript{3} and parenteral\textsuperscript{4,5} glutamine supplementation in VLBW infants could not confirm this finding, possibly because of differences in supplementation method, supplementation dose and definition of infections.

Neonatal infections in VLBW infants are associated with poor neurodevelopmental outcome (including cerebral palsy and visual impairment) in early childhood.\textsuperscript{5,6} We hypothesized that glutamine-enriched enteral nutrition in VLBW infants may lead to improved neurodevelopmental outcome in early childhood by reducing the neonatal infectious morbidity. So far, no reports on long-term effects of early enteral glutamine supplementation are available. In a secondary analysis of a multicenter trial, early administration of parenteral amino acids was found to improve growth at 36 weeks postmenstrual age and to decrease incidence of suboptimal head growth at 18 months of age, associated with poor neurodevelopmental outcome.\textsuperscript{5,59} However, the authors did not provide data about neurodevelopmental outcome in the glutamine-supplemented versus the control group.

The aim of this follow-up study was to evaluate the effect of neonatal glutamine-enriched enteral nutrition in VLBW infants on neurodevelopmental outcome at the corrected age of two years.

Patients and methods

Initial study

The initial study was a randomized double-blind placebo-controlled trial of glutamine-enriched enteral nutrition in 102 VLBW infants (gestational age <32 weeks and/or birth weight <1500 g).\textsuperscript{2} Infants admitted to the level III neonatal intensive care unit (NICU) of the VU University Medical Center, Amsterdam, were eligible for the study. In this study, infants received enteral glutamine supplementation (0.3 g/kg/day) or an isonitrogenous placebo supplementation (alanine) between day 3 and 30 of life. For further details of the study design, including sample size calculation, we refer to the study protocol.\textsuperscript{115} Baseline characteristics of the follow-up cohort and their mothers were recorded in the initial study.\textsuperscript{2}

The national central committee on research involving human subjects and the medical ethical review board of our institute approved the study protocol. All parents gave written informed consent.
Neurodevelopmental Outcome

As part of our routine follow-up program, a pediatric psychologist assessed all participating infants at the corrected age of two years. To assess cognitive development, Mental Development Index (MDI) and Psychomotor Development Index (PDI) of the Bayley Scales of Infant Development (BSID-II) were used.\textsuperscript{132,133} During routine follow-up examination, a pediatrician and a physiotherapist screened the infants for cerebral palsy. Infants with spastic hemiplegia, diplegia, hemidiplegia or quadriplegia were categorized as cerebral palsy. Medical records, including visual and hearing examinations, were reviewed. Both parents and investigators were unaware of treatment allocation in the neonatal period.

Neurodevelopmental impairment was defined as any of the following conditions; MDI ≤ 85 (-1.0 SD), PDI ≤ 85 (-1.0 SD), cerebral palsy, blindness of one or both eyes or hearing loss requiring amplification.

Statistical analysis

Data are presented as mean and standard deviation (SD) and median (range) when appropriate. Infant and maternal characteristics were analyzed by Student’s t-test, Mann-Whitney U test and chi-square test or Fisher’s exact test for continuous normally distributed data, nonparametric continuous data and dichotomous data, respectively.

To adjust for potential confounding variables, data were analyzed by multiple linear or logistic regression for continuous and dichotomous outcome variables, respectively. Adjustments were made for gestational age, birth weight <tenth percentile,\textsuperscript{98} sex, postnatal corticosteroids, and ≥1 neonatal infection.

A p-value <0.05 (two-tailed) was considered significant. SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

Results

At the corrected age of two years, 88/102 (86%) infants were eligible to participate in the follow-up study. Of these 88 infants, 72 (82%) finally participated in the study, of which 40 infants received enteral glutamine supplementation and 32 received placebo supplementation during neonatal period. The complete trial profile is shown in Figure 1. Reasons for not participating in the study were: difficulties with the Dutch language (n=5), family could not be reached (n=4), family did not show up (n=3), migration abroad (n=2), and no informed consent (n=2).

Baseline infant and maternal characteristics were not different in the participating (n=72) and nonparticipating (n=16) groups, except for a higher maternal age and a higher percentage of Caucasian mothers in the participating group (data not shown).
Figure 1. Trial profile

252 infants AD <32 weeks or BW <1500 grams

145 not randomized:
- 48 no informed consent
- 32 participation in other trial
- 29 transfer from extraregional hospital
- 18 transfer <48 hours
- 12 death <48 hrs
- 6 congenital malformations

107 infants randomized

54 glutamine enriched enteral nutrition

2 exclusions:
- 1 morbus Hirschsprung
- 1 multiple congenital malformations

52 analyzed by intention to treat

8 infants died

44 eligible to participate in follow-up study

4 lost to follow-up:
- 2 no informed consent
- 1 difficulties with Dutch language
- 1 migration to foreign country

40 Neurodevelopmental follow-up

53 controls

3 exclusions:
- 1 glutaric acidemia
- 1 trisomy 21
- 1 Fallot’s tetralogy

50 analyzed by intention to treat

- 5 infants died
- 1 chromosomal translocation (found at age 1)

44 eligible to participate in follow-up study

12 lost to follow-up:
- 4 difficulties with Dutch language
- 4 could not be reached
- 3 did not show up at clinic
- 1 migration to foreign country

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Figure 1. Trial profile
Baseline infant and maternal characteristics were not different in the glutamine-supplemented and control group, except for the incidence of ≥1 neonatal infection (Table 1). Mental and psychomotor developmental indices were not different in both groups (Table 2). Adjustment for sex, gestational age, birth weight <tenth percentile, ≥1 neonatal infection and postnatal corticosteroids did not change these results. Neonatal glutamine-enriched enteral nutrition had no effect on the incidence of cerebral palsy or neurodevelopmental impairment (Table 2). None of the infants were blind and the incidence of hearing disability was not different in both groups (glutamine-supplemented and control group: 1/40 (3%) and 1/32 (3%), p=0.87).

Table 1: Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Glutamine-supplemented (n=40)</th>
<th>Control (n=32)</th>
<th>p*</th>
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</thead>
<tbody>
<tr>
<td><strong>Maternal characteristics</strong></td>
<td></td>
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<tr>
<td>Maternal age at birth (years, months)</td>
<td>30.6 (4.6)</td>
<td>28.1 (5.2)</td>
<td>0.17</td>
</tr>
<tr>
<td>Maternal race (% Caucasian)</td>
<td>31/40 (78%)</td>
<td>30/32 (94%)</td>
<td>0.06</td>
</tr>
<tr>
<td>High maternal education†</td>
<td>18/40 (45%)</td>
<td>9/32 (28%)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Infant characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 1 course of antenatal corticosteroids‡</td>
<td>13/40 (33%)</td>
<td>16/32 (50%)</td>
<td>0.13</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>17/40 (43%)</td>
<td>14/32 (44%)</td>
<td>0.92</td>
</tr>
<tr>
<td>Sex: (% Male)</td>
<td>18/40 (45%)</td>
<td>17/32 (53%)</td>
<td>0.49</td>
</tr>
<tr>
<td>Gestational age (wks)</td>
<td>29.4 (1.7)</td>
<td>28.8 (1.6)</td>
<td>0.13</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.18 (0.37)</td>
<td>1.17 (0.31)</td>
<td>0.91</td>
</tr>
<tr>
<td>Birth weight &lt;tenth percentile§</td>
<td>13/40 (33%)</td>
<td>8/32 (25%)</td>
<td>0.49</td>
</tr>
<tr>
<td>5-Minute Apgar score ≤ 6</td>
<td>3/40 (8%)</td>
<td>2/32 (6%)</td>
<td>0.84</td>
</tr>
<tr>
<td>Surfactant</td>
<td>18/40 (45%)</td>
<td>16/32 (50%)</td>
<td>0.67</td>
</tr>
<tr>
<td>PIVH ≥ grade III§</td>
<td>2/40 (5%)</td>
<td>0/32 (0%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Postnatal corticosteroids</td>
<td>3/40 (7%)</td>
<td>2/32 (6%)</td>
<td>0.84</td>
</tr>
<tr>
<td>≥1 neonatal infection¶</td>
<td>22/40 (55%)</td>
<td>25/32 (78%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Age at discharge from NICU (days)</td>
<td>23 (2-122)</td>
<td>28 (3-107)</td>
<td>0.21</td>
</tr>
<tr>
<td>Age at discharge from hospital (days)</td>
<td>60 (35-180)</td>
<td>73 (36-162)</td>
<td>0.19</td>
</tr>
<tr>
<td>Age at follow-up (months)</td>
<td>26 (2.0)</td>
<td>27 (1.3)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Data are mean (SD), median (range) or number (%). *Student’s t test, Mann-Whitney U test and chi-square or Fisher’s exact test for continuous normally distributed, nonparametric continuous and dichotomous data, respectively. †Higher professional or university education.‡One course is 2 doses of 12-mg intramuscular betamethasone, 24 hours apart.§According to Usher.98 ¶All Periventricular Intraventricular Hemorrhage (PIVH) ≥ grade III were diagnosed before start of glutamine supplementation. Sepsis, pneumonia, meningitis, pyelonephritis or arthritis as diagnosed by a combination of clinical signs and a positive culture.127
Discussion

The results of this study indicate that neonatal glutamine-enriched enteral nutrition in VLBW infants has no beneficial effect on neurodevelopmental outcome at age two. No other studies have assessed neurodevelopment after neonatal glutamine supplementation. Positive short-term effects of an intervention in neonatal period might be associated with adverse long-term effects, which underlines the importance of assessment of long-term outcome and safety.6 In our initial study, safety of glutamine-enriched enteral nutrition was assessed by measuring plasma amino acid concentrations at 4 time points. We found that glutamine-enriched enteral nutrition did not alter plasma concentrations of glutamate, a glutamine metabolite that is potentially neurotoxic, or other amino acids.134 In addition, no adverse effects of neonatal glutamine-enriched enteral nutrition were observed in any of the infants.2 Therefore, we concluded that neonatal glutamine-enriched enteral nutrition in a dose of 0.3 g/kg/day is safe in VLBW infants. The absence of adverse neurodevelopmental outcome at the corrected age of two years in VLBW who received neonatal glutamine-enriched enteral nutrition supports this conclusion.

In an additional analysis, we compared infants with a neonatal infection with infants without a neonatal infection. Infants with neonatal infection had lower MDI scores, and more often MDI scores of ≤85, compared to infants without neonatal infection (data not shown). Adjustment for sex, gestational age, birth weight <tenth percentile, neonatal glutamine or placebo supplementation and postnatal corticosteroids did not change these results. The incidence of cerebral palsy and hearing disability was not different in infants with or without neonatal
infection. However, the risk for neurodevelopmental impairment was higher in infants with neonatal infection than in infants without neonatal infection (p<0.05) These findings are in line with results of several other studies. To develop strategies to improve long-term outcome, understanding the pathophysiology of central nervous injury in VLBW infants is important. From various studies, it is now clear that infections such as chorioamnionitis, sepsis and necrotizing enterocolitis have an adverse effect on neurodevelopmental outcome, due to secondary brain damage.

The question remains why the adverse effect of a neonatal infection on neurodevelopmental outcome is not completely counteracted by the anti-infectious effect of glutamine supplementation. A possible explanation could be a difference in severity of infections between glutamine-supplemented and control groups, i.e. more severe infections in the glutamine-supplemented group. This explanation seems unlikely, because the two groups did not differ in type of pathogen, type of infection nor in short term outcome (data not shown). Another explanation might be that although neonatal glutamine-enriched enteral nutrition decreases the incidence of neonatal infections, it may also have a negative effect on neurodevelopmental outcome. However, as we found no differences in neurodevelopmental outcome in glutamine-supplemented infants versus controls without neonatal infections, this explanation is unlikely.

Some aspects of our study design need to be addressed. Firstly, we recognize that a control group without glutamine or alanine supplementation would have been more representative for daily practice. However, comparing glutamine supplementation with no amino acid supplementation limits the possibility of drawing conclusions on the effect of glutamine, as the results may reflect the effect of amino acid supplementation per se. Therefore, in the initial study, we decided to compare glutamine supplementation with isonitrogenous alanine supplementation. Secondly, as the sample size calculation of the study was based on the primary outcome of the initial trial, the sample size of the follow-up study was relatively small. As a consequence, the conclusions of our study are susceptible to a type II error. Thirdly, in the initial study, the rate of neonatal infections was relatively high in both treatment groups. However, the relatively high rate of neonatal infections is in line with the results of a recent surveillance study of nosocomial infections in our NICU. We used predefined definitions for nosocomial infections in neonates that were adapted from the current definitions from the Center for Disease Control and Prevention for nosocomial infections in children < 1 year. The relatively high infection rate can be partially explained by our definitions of nosocomial infections and the relatively high device-associated utilization ratios in our NICU. Fourthly, in our initial study, all cases of severe PIVH (≥ grade III) were randomly assigned in the glutamine-supplemented. However, all cases of PIVH ≥ grade III were diagnosed before the start of glutamine supplementation. As 3 cases with PIVH ≥ grade III died and 1 was lost to follow-up, only 2 were included in the follow-up study. Addition of PIVH to our regression analysis did not influence our results (data not shown). Finally, the predictive value of the Bayley scales for cognitive function has been criticized before. Neurodevelopmental outcome of infants may change, as the infants get
older. Therefore, further follow-up study of this cohort may contribute to better understanding whether these effects are transient or permanent.

In conclusion, this is the first study into the effects of neonatal glutamine-enriched enteral nutrition in VLBW infants on neurodevelopmental outcome at the corrected age of two years. Our study indicates that neonatal glutamine-enriched enteral nutrition in VLBW infants does not improve neurodevelopmental outcome at the corrected age of two years. Based on the absence of adverse neurodevelopmental outcome, neonatal glutamine-enriched enteral nutrition in VLBW infants seems to be safe. In line with other studies, we found that neonatal infections in VLBW infants are associated with adverse neurodevelopmental outcome.\textsuperscript{6,57,136-138} Further follow-up study of this well-defined cohort of VLBW infants may contribute to a better understanding of long-term effects of neonatal glutamine-enriched enteral nutrition on neurodevelopmental outcome.