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Introduction

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Mechanisms of glutamine action in very low birth weight infants

Experimental studies have shown that the amino acid glutamine plays an important role in maintaining the functional integrity of the gut. Glutamine serves as fuel for enterocytes, and provides nitrogen for the synthesis of amino sugars, that are involved in the maintenance of tight junctions, and mucin synthesis. Moreover, glutamine has stimulatory and regulatory effects on mucosal cell proliferation and differentiation. In addition, glutamine is used at a high rate by cells of the immune system, as a source of energy, a precursor for protein synthesis and a donor of nitrogen for the synthesis of pyrimidines, nucleotides and amino sugars. In very low birth weight (VLBW) infants, the mechanisms of glutamine action have been hardly investigated. Only, a study of Neu et al. showed that decreased infectious morbidity in VLBW infants was associated with changes in immune-cell type distribution.

a. Intestinal permeability
One suggested mechanism of the beneficial effect of glutamine is its role in maintaining functional integrity of the gut. Experimental studies have shown that glutamine supplementation reduced intestinal atrophy and decreased intestinal permeability following parenteral nutrition, abdominal radiation, and pharmacological agents. Studies of glutamine supplementation in adults have found varying effects on intestinal permeability and integrity of the intestinal epithelium. In VLBW infants, the intestinal permeability decreases during the first days of life as part of the postnatal adaptation of the gut. Until now, the effect of enteral glutamine supplementation on this process has not been studied.

b. Intestinal microflora
Another aspect of the postnatal adaptation of the gut is the development of the intestinal microflora. In VLBW infants, the intestinal colonization by health promoting bacteria (e.g. Bifidobacterium and Lactobacillus species) is delayed, as compared to healthy breast-fed infants. The mucus layer is an important site for bacterial colonization and its composition may modulate bacterial adherence. A study in rats on parenteral nutrition suggested that glutamine supplementation improved thickness and optical density of the mucus gel. These glutamine-mediated changes of the mucus layer in turn may lead to altered bacterial adherence and colonization of the gut. To our knowledge, the effect of enteral glutamine supplementation on the development of the intestinal microflora in VLBW infants has not been investigated.

c. Cytokine responses
Glutamine is used at a high rate by cells of the immune system, as a source of energy, a precursor for protein synthesis and a donor of nitrogen for the synthesis of pyrimidines, nucleotides and amino sugars. The presence of glutamine in vitro
increases the cytokine production of T-lymphocytes following stimulation with various mitogens. A subset of T-helper cells known as T helper type 1 (Th1) cells, is implicated in the cell-mediated resistance to infections, whereas Th2 cells are involved in the regulation of antibody responses. The Th subsets can be differentiated based on the production of a specific panel of cytokines. It has been shown that glutamine supplementation increases the Th1 response in septic mice and in adult trauma patients. The effect of glutamine supplementation on cytokine responses in VLBW infants is unknown. Enhancement of antimicrobial activity of the immune system may be an explanation for a beneficial effect of enteral glutamine supplementation on infectious morbidity.

**Glutamine supplementation in very low birth weight infants**

In critically ill adult patients, glutamine is considered a conditionally essential amino acid as endogenous glutamine synthesis may not meet the increased demand. VLBW infants may be especially susceptible to glutamine depletion because placental supply suddenly ceases at birth, tolerance of enteral nutrition is limited and parenteral nutrition does not contain glutamine for solubility and stability reasons. Glutamine depletion has negative effects on the functional integrity of the gut and leads to immunosuppression. Although VLBW infants are able to synthesize glutamine de novo, the question arises whether in the immediate postnatal period, systemically available glutamine meets the demands of organs with a high glutamine consumption, e.g. the gut. In addition, several experimental studies have shown that supply of glutamine from the apical side is of critical importance for maintaining intestinal integrity.

Trials of both parenteral and enteral glutamine supplementation in VLBW infants have found varying results. Three single-center studies in VLBW infants have shown a positive effect of glutamine supplementation on feeding tolerance and the incidence of infectious morbidity. However, 2 recent multicenter trials did not find decreased infectious morbidity in VLBW infants receiving parenteral or enteral glutamine supplementation. Nevertheless, in the latter study, feeding tolerance was improved in the glutamine-supplemented group.

Although many human studies evaluated the efficacy of glutamine supplementation, only few studies specifically addressed its safety. One aspect of safety is the effect of glutamine supplementation on plasma amino acid concentrations. Plasma amino acid concentrations were presented in several studies of glutamine supplementation in VLBW infants. Plasma amino acid concentrations may provide valuable information about the potentially toxic effects of glutamine, that have been proposed by Garlick in a review on glutamine safety. Other aspects of safety include mortality and the neurodevelopmental outcome.
Follow-up

Several studies in VLBW infants have investigated the effect of parenteral or enteral glutamine supplementation on morbidity and outcome in the neonatal period.\textsuperscript{8,39-42} However, little is known about the long-term effects of this nutritional intervention. Knowledge about the effect of nutritional interventions on long-term outcome and disease later in life (e.g. allergy and asthma) may contribute to deliberate choices in neonatal nutritional support in VLBW infants, as recently discussed in a workshop on research issues in neonatology.\textsuperscript{47}

As during pregnancy the maternal immune response is skewed towards Th2 immunity,\textsuperscript{48,49} Th2 cytokine responses dominate the neonatal immune response.\textsuperscript{50} After birth, microbial exposure stimulates Th1 cytokine responses and deviates the neonatal immune response towards balanced Th1/Th2 cytokine responses.\textsuperscript{50} Delayed transition from fetal Th2-polarized cytokine responses to adult Th1-polarized cytokine responses may lead to long-term dysregulation of Th2 responses\textsuperscript{51} and allergic diseases.\textsuperscript{52-53} Factors early in life, such as mode of delivery and nutrition may influence the shift from Th2 cytokine towards balanced Th1/Th2 cytokine responses.\textsuperscript{54-58} In studies in septic mice\textsuperscript{32} and adult trauma patients\textsuperscript{33} glutamine supplementation increased the Th1 response. We hypothesized that glutamine supplementation in VLBW infants enhances the maturation of the immune response by stimulating Th1 cytokine responses and possibly leads to decreased allergic diseases later in life. Besides a decrease in allergic diseases later in life, glutamine supplementation in VLBW infants may affect the long-term resistance against infectious diseases.

Outline of thesis

The primary aim of this thesis is to investigate the effect of glutamine-enriched enteral nutrition on clinical outcome in VLBW infants. Furthermore, the role of glutamine-enriched enteral nutrition in postnatal adaptation of the gut and modulation of the immune response is investigated. Finally, the focus is on the long-term effect of glutamine-enriched enteral nutrition in VLBW infants.

In chapter 2, we describe a double-blind randomized controlled trial, that is designed to determine the effect of glutamine-enriched enteral nutrition on feeding tolerance in VLBW infants. Furthermore, infectious morbidity and short-term outcome are evaluated. To assess safety of glutamine-enriched enteral nutrition in VLBW infants, we have determined plasma amino acid concentrations during the course of aforementioned trial (chapter 3).

As part of the previously described trial, we have performed several studies to elucidate the role of glutamine in postnatal adaptation of the gut and modulation of the immune response. In chapter 4, we present a study that investigates the effect of
glutamine-enriched enteral nutrition on the functional integrity of the gut, as reflected by intestinal permeability. In chapter 5-I and 5-II, we focus on the development of the intestinal microflora. First, we present a study of the literature on the development of the intestinal microflora in VLBW infants. Second, we describe the effect of glutamine-enriched enteral nutrition on the development of the intestinal microflora in these infants. In chapter 6, we describe the results of a study into the effect of glutamine-enriched enteral nutrition on neonatal cytokine responses.

In chapter 7, we present a follow-up study, that evaluates the effect of glutamine-enriched enteral nutrition in VLBW infants on the incidence of allergic and infectious diseases during the first year of life.

In chapter 8, we discuss the results of the studies and the implications for future research. Finally a summary of the thesis is provided.