Fresh-Frozen Plasma as a Source of Exogenous Insulin-Like Growth Factor-I in the Extremely Preterm Infant

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Context: Preterm birth is followed by a decrease in circulatory levels of IGF-I and IGF binding protein (IGFBP)-3, proteins with important neurogenic and angiogenic properties.

Objective: Our objective was to evaluate the effects of iv administration of fresh-frozen plasma (FFP) from adult donors on circulatory levels of IGF-I and IGFBP-3 in extremely preterm infants.

Design, Setting, and Patients: A prospective cohort study was performed in 20 extremely preterm infants [mean (SD) gestational age 25.3 (1.3) wk] with clinical requirement of FFP during the first postnatal week. Sampling was performed before initiation of transfusion, directly after, and at 6, 12, 24, and 48 h after completed FFP transfusion.

Main Outcome Measures: Concentrations of IGF-I and IGFBP-3 before and after transfusion of FFP were determined.

Results: FFP with a mean (so) volume of 11 ml/kg (3.1) was administered at a median postnatal age of 2 d (range 1–7). Mean (so) IGF-I and IGFBP-3 concentrations in administered FFP were 130 (39) and 2840 μg/liter (615), respectively. Immediately after FFP transfusion, mean (so) concentrations of IGF-I increased by 133% from 11 (6.4) to 25 μg/liter (9.3) (P < 0.001) and IGFBP-3 by 61% from 815 (451) to 1311 μg/liter (508) (P < 0.001). Concentrations of IGF-I and IGFBP-3 remained higher at 6 (P < 0.001, P = 0.009) and 12 h (P = 0.017, P = 0.018), respectively, as compared with concentrations before FFP transfusion. Typical half-life of administrated IGF-I was 3.4 h for a 1-kg infant.

Conclusion: Transfusion of FFP to extremely preterm infants during the first postnatal week elevates levels of IGF-I and IGFBP-3.

Very preterm infants have decreased volumes of both cerebral white and gray matter, as well as delayed maturation of white matter tracts, when examined with magnetic resonance imaging at term or during later childhood, and these findings have been associated with adverse neurodevelopmental outcome (1–3). IGF-I is a fetal growth factor essential for the development of the central nervous system. IGF-I promotes proliferation, maturation, and differentiation of neural stem cells, and of neuronal and oligodendrocyte precursors (4). After induced malnutrition, asphyxia, or inflammation, IGF-I has been demonstrated to have neuroprotective properties both in vivo and in vitro (5–8). In addition, IGF-I has played an important role for retinal vascular development in both experimental and clinical studies (9, 10). Bioavailability of IGF-I is regulated by several binding proteins, in which IGF binding protein (IGFBP)-3 is the dominating binding protein. IGF-I is primarily maintained within the circu-
level by complex binding to IGFBP-3 and the acid-labile subunit (ALS) (11). Dissociation between IGF-I and IGFBP-3 is controlled by proteases, which regulate tissue availability of bioactive free IGF-I, capable of binding to cellular IGF-receptors. IGFBP-3 can modulate cellular mechanisms independently of IGF-I. Recently, IGFBP-3 has promoted vascular regrowth in experimentally induced retinopathy (12).

We have previously shown that concentrations of both IGF-I and IGFBP-3 decrease rapidly during the first postnatal days after very preterm birth (13). Persisting low levels of IGF-I have been associated with retinopathy of prematurity and slow weight gain, as well as slow head growth in preterm infants (9, 14, 15).

An attempt to maintain circulating levels of IGF-I of the preterm infant to levels similar to those of the fetus in utero by early optimized nutrition or other interventions may support normal neural and vascular growth, protect against exogenously induced inflammatory or asphyxial events, and have subsequent positive effects on the development of the central nervous system (16).

Fresh-frozen plasma (FFP) is used as volume expansion or as a source of coagulation factors in neonatal intensive care. Plasma from healthy adults contains IGF-I and IGFBP-3 in considerably higher concentrations than those reported for very preterm infants, and these proteins have been resistant to freeze-thaw treatment before transfusion (17).

We hypothesized that iv administration of FFP from adult donors to preterm infants would increase their circulating levels of IGF-I and IGFBP-3. This paper describes a study of 20 extremely preterm infants in whom we evaluated the effect of administered FFP during the first postnatal week on circulating levels of IGF-I and IGFBP-3.

Subjects and Methods
Study population
The study was a prospective cohort study (December 2005-July 2006) of 20 preterm infants treated at the Neonatal Intensive Care Units in Lund University Hospital or Queen Silvia Children Hospital, Gothenburg. The study protocol was approved by the Regional Committee for Research Ethics, Lund. All pregnancies were dated by ultrasound at 17–18 gestational weeks.

Inclusion criteria were a gestational age less than 28 wk at birth, a postnatal age less than 7 d, the requirement of FFP for clinical purposes, and informed parental consent from both parents. An arterial catheter was put on ice, and delivered within 20 min to the local chemical laboratory. Serum was obtained from the blood samples after spin drying and was thereafter stored together with samples from FFP at −70 °C until assayed.

Plasma-glucose concentrations in neonatal blood were analyzed before transfusion, immediately after, and at 6 h after completed FFP transfusion (ABL 735; Radiometer, Copenhagen, Denmark).

Clinical data
Antenatal data were obtained from maternal records. The total number of doses of antenatal steroids was registered. Relevant neonatal data were obtained from the infant’s records in which gender, gestational age at birth, birth weight, and Apgar score were registered. The infants were defined as small for gestational age if the deviation in birth weight was more than 2 SD below the gestational age-related mean of the population (18). Septicemia was defined as a positive blood culture with increased C-reactive protein level (>5 mg/liter) and clinical signs of infection at birth or at study entry.

The accumulated dose of hydrocortisone (mg/kg) or insulin (U/kg) administered during the last 24 h before study entry (time point when FFP transfusion was initiated) was registered. Packed red blood cells (ml/kg) or FFP (ml/kg) administered on clinical indication during 24 h before study entry and during the following 48 h were registered. Intravenous glucose intakes (mg/kg·min) during 6 h before, during FFP infusion, and the following 6 h were registered.

Quantitative analysis of IGF-I and IGFBP-3
IGF-I and IGFBP-3 concentrations were analyzed using IGFBP-blocked RIA and a specific RIA (Mediagnost GmbH, Tübingen, Germany). The IGF-I samples were diluted 1:50, and the IGFBP-3 samples were diluted 1:300. The intraassay coefficients of variation for IGF-I were 18, 11, and 7% at concentrations of 9, 33, and 179 µg/liter, and for IGFBP-3 10, 7, and 6% at concentrations of 716, 1750, and 3929 µg/liter, respectively. All samples were analyzed within the same assay. The methods have been described in detail previously (19).

Pharmacokinetic profile of IGF-I
Estimation of pharmacokinetic parameters of IGF-I was performed by population analysis, using nonlinear mixed effects modeling in the computer program NONMEM (20). The method estimates typical values in the study population and between-subject variability in the parameters were evaluated. Volume of distribution (Vd) and clearance (Cl) were scaled allometrically by body weight (BW) and calculated as: Vd = Vd1 kg × BW (kg) and Cl = Cl1 kg × BW (kg)0.75, respectively. Calculation of half-life (t1/2) was based on the equation ln(t1/2) = ln (2) Vd/Cl.

Statistical analysis
Statistical analysis was performed using SPSS version 15.0 for Microsoft Windows (SPSS, Inc., Chicago, IL). The effect of administration of FFP on concentrations of IGF-I and IGFBP-3 were primarily evaluated using ANOVA for repeated measures. Differences between concentrations at specific time points were assessed using the paired t test. Relationships between continuous or categorical variables and IGF-I and IGFBP-3 concentrations were assessed using Pearson’s correlation or the unpaired t test as appropriate.

Results
There were 20 preterm infants included in the study. The mean (SD) gestational age was 25.3 wk (1.3) and birth weight 724 g (177). There were 13 infants (65%) that were boys, and 10 infants (50%) were small for gestational age. The mothers of all infants except one received antenatal treatment with a median of
two doses (range one to two) of betamethasone (12 mg/dose). No infants had sepsis at birth or before study entry. One infant was treated with one dose of hydrocortisone (0.9 mg/kg) and another infant with infusion of insulin (total dose 2 U/kg); both treatments were discontinued before study entry.

FFP was administered to the infants at a median postnatal age of 2 d (range 1–7). The mean (sd) administered volume of FFP was 11 ml/kg (3.1) during a median of 120 min (range 90–240). Mean (sd) IGF-I and IGFBP-3 concentrations in administered plasma were 130 (39) and 2840 μg/liter (615), corresponding to a mean (sd) total administered amount of IGF-I and IGFBP-3 of 1.4 (0.5) and 31 μg/kg (11.5), respectively.

Mean (SEM) serum concentrations of IGF-I, IGFBP-3, and an IGF-I to IGFBP-3 ratio at the respective sampling time points are illustrated in Fig. 1. Administration of FFP had a significant effect on concentrations of IGF-I and IGFBP-3, as evaluated by ANOVA for repeated measures (P < 0.001). Immediately after completed administration of FFP, mean (sd) concentrations of IGF-I increased by 133% from 11 (6.4) to 25 μg/liter (9.3) (P < 0.001). Concentrations of IGF-I remained higher at 6 h, 16 μg/liter (6.2) (P < 0.001), and at 12 h, 14 μg/liter (7.0) (P = 0.017) after completed transfusion, but not thereafter.

Mean (sd) corresponding concentrations of IGFBP-3 increased by 61% from 815 (451) to 1311 μg/liter (508) (P < 0.001) immediately after FFP transfusion and remained higher at 6 h, 986 μg/liter (333) (P = 0.009), and at 12 h, 891 μg/liter (406) (P = 0.018) after completed transfusion, but not thereafter.

Mean (sd) ratio between IGF-I and IGFBP-3 concentrations in administered plasma was 0.05 (0.01). Mean (sd) IGF-I to IGFBP-3 ratio increased by 44% from 0.014 (0.006) to 0.02 (0.008) (P = 0.001) immediately after FFP transfusion with no significant change observed thereafter, compared with levels before FFP transfusion. Concentrations of IGF-I and IGFBP-3 obtained subsequently to additional FFP administered on clinical indications during the study period were excluded from the analysis previously described.

Infant gender, gestational age, birth weight, birth weight for gestational age or postnatal age were neither associated with concentrations of IGF-I, IGFBP-3, and the ratio of IGF-I to IGFBP-3 before or after FFP, nor with corresponding relative changes in concentrations.

Amounts of IGF-I and IGFBP-3 (μg) present in the circulation after completed FFP transfusion were calculated using the formula: concentration of protein (μg/liter) after completed FFP × estimated plasma volume (50 ml/kg + administered volume of FFP) × 1000 − concentration of protein (μg/liter) before FFP × estimated plasma volume (50 ml/kg) × 1000.

Amount of administered IGF-I (μg) in FFP [volume of administered FFP (ml) × concentration of protein in FFP (μg/liter)/1000] correlated with the amount of remaining IGF-I (μg) after completed FFP transfusion (r = 0.81; P = 0.000). The corresponding correlation for administered IGFBP-3 (μg) vs. remaining IGFBP-3 (μg) was r = 0.70; P = 0.001.

The ratio between the amount of IGF-I present after completed FFP transfusion and administered amount of IGF-I (ranged between 40 and 103%) correlated positively to gestational age at birth (r = 0.68; P = 0.001). This correlation re-
mained significant after adjustment for postnatal age at FFP transfusion, duration of FFP transfusion, and BW for gestational age. No corresponding relationships were observed for IGFBP-3.

For calculation of $t_{1/2}$, Vd, and Cl of IGF-I, infants who had received FFP before study entry were excluded from analysis. In addition, samples collected after additional FFP during the study protocol were omitted. A one-compartment model with an estimated endogenous concentration described the 86 samples from the 15 infants adequately. Typical $t_{1/2}$ of IGF-I for a neonate of 1 kg was 3.4 h based on typical estimates [relative se (RSE)] of 90 ml/kg (14%) for Vd and 18 ml/h/kg (22%) for Cl. Estimated endogenous concentration of IGF-I was 9.6 μg/liter (RSE 11%). Between-subject variability was only supported in the endogenous concentration with an estimated coefficient of variation of 39% (RSE 18%). The proportional residual error was 32% (RSE 7.6%). The model predicted a typical $t_{1/2}$ of IGF-I for a neonate of 0.5 and 1.5 kg to 2.9 and 3.8 h, respectively.

Five infants (25%) received a mean (SD) FFP amount of 12.5 ml/kg (2.5) during the last 24 h before study entry. These infants had a tendency toward higher concentrations of IGF-I at study entry (15.4 vs. 9.1 μg/liter; $P = 0.05$) and higher concentrations of IGFBP-3 (1404 vs. 604 μg/liter; $P < 0.001$), compared with infants who had not received FFP during the last 24 h before study entry. Transfusions of packed erythrocytes administered to seven (35%) infants during the corresponding time period were not associated with levels of IGF-I or IGFBP-3 at study entry.

Four infants (20%) received an additional transfusion of FFP due to clinical indications at study sampling between 12 and 24 h after completed FFP administration. Mean concentrations of IGF-I at 24 h were higher in infants who received an additional FFP transfusion, mean (SD) 26 μg/liter (3.5), compared with the remaining infants, 11 (6.5) μg/liter ($P < 0.001$). Mean (SD) corresponding concentrations of IGFBP-3 at 24 h were 1701 μg/liter (638) and 750 μg/liter (372) ($P = 0.001$).

A high proportion of infants 18 of 20 (90%) received packed erythrocytes after study entry. Volume of administered packed erythrocytes (ml/kg) during the study period did not correlate with concentrations of IGF-I or IGFBP-3 at any time point.

Mean (SD) plasma glucose concentrations before, directly after, and at 6 h after FFP transfusion were 6.2 (2.2), 6.5 (2.0), and 6.9 mmol/liter (2.7), respectively. Plasma glucose concentrations did not differ immediately after FFP or at 6 h, compared with levels before transfusion. No difference was observed in iv glucose intake (mg/kg · min) between the three analyzed time periods, during 6 h before FFP transfusion, during FFP transfusion, and during 6 h after transfusion.

**Discussion**

This study shows that administration of FFP from adult donors to extremely preterm infants during the first postnatal week significantly elevates circulatory levels of IGF-I as well as IGFBP-3. These findings suggest that exogenous administration of IGF-I may be one way of counterbalancing the immediate decrease in IGF-I and IGFBP-3, which has been described after very preterm birth (13).

Serum concentrations of IGF-I more than doubled after transfusion of FFP and thereby reached levels close to those described as physiological in the fetus at similar gestational ages (13, 21, 22). This was achieved by a relatively low administered dose, on average 1.4 μg/kg, compared with iv doses administered in pharmacokinetic studies of recombinant human (rh) IGF-I in healthy adults (23–25). The sufficiency of such a low dose is to be expected because both baseline and resulting concentrations after FFP in these extremely preterm infants were more than 10-fold lower than those described in adults. Mean concentration of IGF-I at study entry was 11 μg/liter, which corresponds well to previously described IGF-I concentrations in early postnatal blood in very preterm infants (13).

We observed a good correlation between calculated administered and received amount of both IGF-I and IGFBP-3. The variation in concentrations of IGF-I in administered FFP (50–210 μg/liter) and in administered volume of FFP enabled an assessment of resulting serum concentrations of IGF-I after administration of IGF-I over a range of doses. The data suggest that an iv dose between 1 and 2 μg/kg IGF-I is required to achieve physiological concentrations of IGF-I during the immediate postnatal period in extremely preterm infants.

Although concentrations of IGF-I and IGFBP-3 immediately after FFP transfusion achieved targeted physiological levels, the subsequent decline in concentrations was rapid. Concentrations of both proteins at 24 h after transfusion of FFP were similar to baseline levels. The half-life of IGF-I was considerably shorter than in adults in whom a half-life of total IGF-I after iv administration of rhIGF-I was calculated to be about 30 h (25). This suggests that administration of IGF-I in preterm infants should be based on continuous infusion of IGF-I to achieve targeted physiological concentrations during sustained time periods.

The ratio between the calculated amount of IGF-I (μg) present in the circulation after completed FFP transfusion and administrated IGF-I (μg) varied between 40 and 100%, and was used as an estimate of the proportion of IGF-I retained within the circulation. The ratio correlated positively with gestational age, i.e., the most mature infants had the highest proportion of IGF-I retained within the circulation, suggesting that the elimination rate from the circulation increased with decreasing gestational age. The calculation of the remaining amount in the circulation was based on an assumption of a plasma volume of 50 ml/kg in all infants. Because measured plasma volume varies over a wide range in both preterm and term infants, the present results should be considered as approximative (26). The described association between increasing prematurity and increased elimination of IGF-I is further supported by the finding that lower BW was associated with shorter $t_{1/2}$.

The IGF-I to IGFBP-3 ratio has been used as an estimate of the availability of nonprotein-bound IGF-I with an increased ratio suggesting an increased availability of free bioactive IGF-I. A higher IGF-I to IGFBP-3 ratio during the first postnatal month has been associated with higher growth velocity in moderately preterm, compared with term infants (27). The IGF-I to IGFBP-3 ratio increased after FFP transfusion, which indicates an increase
in the nonprotein bound portion of free IGF-I available for tissue uptake. The increase was explained by a significantly higher IGF-I to IGFBP-3 ratio in administered FFP than in baseline samples from infants.

The majority of IGF-I in administrated FFP would be expected to be complex-bound in a ternary complex with IGFBP-3 and ALS, which theoretically would prolong the half-life of circulatory IGF-I compared with administration of nonprotein bound IGF-I (28). The reason for the observed rapid decrease in concentrations of both IGF-I and IGFBP-3 is not clear. The infants were studied at an early postnatal age (median 2 d). We have previously described a severe decrease between cord blood levels of IGF-I and those at 72 h after very preterm birth, which suggested a low or absent endogenous production of IGF-I during the immediate postnatal period (13). This observation is supported by a very slow increase in levels of IGF-I during the first postnatal weeks in very preterm infants (9). Low or absent endogenous production of IGF-I may in part explain the rapid decrease in concentrations, compared with that observed in adults as mentioned previously.

Very preterm birth is associated with a circulating pro-inflammatory response in a large proportion of infants (29). Both acute and chronic inflammation have been associated with a decrease in levels of IGF-I and of IGFBP-3 (13, 30). In addition, pro-inflammation may induce GH insensitivity (31). Preterm infants have displayed elevated levels of GH and low levels of IGF-I, and, importantly, an association between increase in GH and subsequent morbidity, i.e. retinopathy of prematurity (32). Alterations in levels of GH in response to supplementation with IGF-I would be essential to evaluate. This was not performed due to restricted blood volumes and must be addressed in future study.

The affinity between IGFBP-3 and IGF-I is regulated by specific proteases (11). Up-regulation of proteases is induced by pro-inflammatory and catabolic states (30, 33). Extremely preterm infants are catabolic during the initial postnatal period due to an insufficient nutrient supply that does not achieve intrauterine requirements for normal fetal growth (34). The significance of proteases in extremely preterm infants is yet to be determined because fetal serum has not been shown to demonstrate increased IGFBP-3 protease activity (35). Hypothetically, both pro-inflammation and catabolism with an increased proteolytic degradation of IGFBP-3 may have accentuated the observed decrease in concentrations of IGF-I and IGFBP-3.

Proteins normally present in adult human plasma such as transferrin and plasminogen have been observed to interfere with the binding affinity of IGFBP-3 (36, 37). High cytokine levels in plasma have been associated with febrile transfusion reactions (38). The risk of blood-borne infections is very small, but still not negligible. Therefore, administration of a rhIGF/IGFBP-3 complex in donor plasma is presumably present in ternary complex bound to both IGFBP-3 and ALS.

Administration of rhIGF-I has been associated with hypoglycemia. IGF-I exists in the circulation at molar concentrations that are about 1000 times higher than for insulin and can bind to the insulin receptor, which implies that free IGF-I has the capacity of interfering with glucose metabolism (28). We measured glucose concentrations before and after administration of FFP. Although both circulatory levels of IGF-I and the IGF-I to IGFBP-3 ratio increased substantially after FFP transfusion, we did not observe any significant changes in glucose concentrations. All infants received continuous glucose infusion that may have counteracted the development of hypoglycemia. Intravenous infusion of IGF-I in ovine fetuses has been shown to decrease insulin concentrations with glucose concentrations remaining unchanged. This may be one mechanism whereby hypoglycemia is avoided (39). The presence of IGFBPs in the circulation may limit the hypoglycemic side effects of free IGF-I (40).

In conclusion, we have shown that infusion of FFP elevates IGF-I and IGFBP-3 in extremely preterm infants. The results may serve as a basis for further studies on the role of IGF-I and IGFBP-3 as neuroprotective and vasoprotective agents in extremely preterm infants.

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