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Effects of Intralipid infusion on hemorheology and peripheral resistance in neonates and children

Abstract Deleterious microcirculatory effects of Intralipid (IL) infusion may be caused by hemorheological or vascular effects. The aim of this investigation was to study vascular and hemorheological effects of IL in preterm and fullterm neonates and children. Ten preterm newborns, 10 fullterm neonates, and 10 children received an initial infusion of IL (0.6 g/kg) over 4 h. Calf blood flow (venous occlusion plethysmography), blood pressure (Dinamap), whole blood and plasma viscosity (capillary viscometer), red blood cell deformability (rheoscope), and erythrocyte aggregation (aggregometer) were measured before and after administration of IL. Plasma triglyceride levels showed the greatest increase in preterm infants. Whole blood viscosity decreased by about 10% in all three groups because of a similar reduction in hematocrit. Red blood cell aggregation decreased by about 20% after IL infusion. Blood pressure rose by 10%, and peripheral blood flow declined by about 10% in the three groups. Vascular hindrance, a calculation of blood pressure divided by blood flow and viscosity, was raised by about 20%, suggesting marked vasoconstriction of peripheral arteries. Vasoconstriction rather than hemorheological changes during infusion of IL may play a crucial role in the pathogenesis of circulatory alterations in parenterally-fed neonates.

Keywords Children · Circulation · Hemorheology · Intralipid · Newborn

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Introduction

Intravenous lipid emulsions of soybean triglycerides, such as Intralipid (IL), are principally given to prevent deficiency of essential fatty acids, to supply the necessary calories for energy expenditure and growth, and to improve the support of fat-soluble vitamins in patients of all ages, including newborn infants.

Besides various metabolic side effects of fat emulsions in neonates, several papers report pulmonary complications in preterm infants as a result of IL infusion, including fat embolism [1, 2], increased pulmonary vascular resistance [3, 4], increased alveolar–arteriolar oxygen gradient [5], and increased risk of bronchopulmonary dysplasia [6, 7]. These observations have been connected with lipid accretion in pulmonary microvessels [2] and with several mechanisms that influence microcirculation, such as lipid peroxide production [8], increased production of prostaglandins and thromboxane [9, 10], and diminished bioavailability of the endothelium-derived vascular relaxant NO [11]. Thus, a relationship between IL infusion and microcirculatory deterioration in neonates seems probable, although clinical impact and mechanisms remain unclear.

In healthy adults, IL infusion has resulted in a marked rise in systolic arterial blood pressure [12] and peripheral [12] and systemic [13] vascular resistance. It has been suggested that alterations of hemorheological properties through hypertriglyceridemia caused by IL may be a causative factor in the rise of peripheral resistance.

An increase in either vascular reactivity or blood viscosity caused by a fast rise in plasma triglycerides may contribute to the circulatory effects of IL infusions according to the Hagen–Poiseuille law [14]. There are only few and partially differing results about the effects of IL infusion on blood viscosity and its determinants plasma viscosity, red blood cell (RBC) aggregation, and RBC deformability [15–17].

In preterm and small-for-gestational-age newborns, intravenous fat elevates the concentrations of triglyce-

rides and free fatty acids significantly more than in fullterm neonates, children, or adults [18, 19]. These findings prompted us to determine blood viscosity and its determinants (hematocrit, plasma viscosity, and RBC aggregation and deformabililty) as well as vascular hindrance in preterm newborns, fullterm neonates, and children before and after IL infusion.

Patients and methods

Patients and blood samples

Effects of parenterally administered IL were studied in 10 preterm infants (gestational age 26–32 weeks, birth weight 1,000–2,300 g), 10 fullterm neonates (gestational age 38–41 weeks, birth weight 2,900–3,600 g), and 10 children (age 0.5–12 years). Excluded were infants and children with signs of septicemia, arterial hypotension, pulmonary hypertension, or cardiac or renal disease, as well as infants and children with serum bilirubin > 12 mg/dl or inspiratory oxygen > 40%. None was being treated with sedatives, analgesics, diuretics, or vasoactive drugs. In the preterm and fullterm neonates, IL was started at 48–72 h of postnatal age because they had not tolerated oral feeding since birth. The children were given IL because they had not tolerated oral feeding 48–72 h after an operation. All infants and children were studied immediately before and after they received an initial total dose of 6 ml/kg of IL 10% over 4 h. Blood samples were taken before and at the end of the infusion.

Hemodynamic measurements

Bilateral calf blood flow was measured by venous occlusion plethysmography using calibrated mercury in silastic strain gauges. Blood pressure was assessed via oscillometry (Dinamap Pro Monitor 300, Criticon, Tampa, USA). In plethysmography and in blood pressure measurements, an average of five recordings was used for analysis.

Hematologic methods

Hematocrit was determined by a micro-hematocrit method. The values were corrected for 2% of trapped plasma. RBC count, hemoglobin concentration, and white cell count were determined using a Coulter Counter (Coulter Electronics, Harpenden, Herts, UK). Total plasma protein concentration was measured by the Biuret test. Plasma fibrinogen, immunoglobulin G, and albumin concentrations were determined via radial-immunodiffusion techniques (M-Partigen kits, Behring, Marburg, Germany).

Hemorheological methods

All hemorheological measurements were made within 1 h after blood collection. Blood and plasma viscosities were determined by means of a capillary viscometer [20]. A tube with a diameter of 100 μm and length of 1 cm was perfused with whole blood and plasma at a temperature of 37° and a pressure of 25 cm H_2O. Blood and plasma viscosities (11) were calculated from the passage times of the samples (ts) and distilled water (tH_2O) and from the viscosity of water at 37° (0.6915 mPas):

$$\eta = (ts/tH_2O)(0.6915).$$

Relative viscosity was calculated as ratio of blood to plasma viscosity.

RBC aggregation was assessed at 22°C using the Myrenne Erythrocyte Aggregometer MA2 (Myrenne, Roetgen, Germany), which consists of a transparent cone plate viscometer [21]. A blood sample with an adjusted hematocrit of 40% is sheared for 20 s to disperse all RBC aggregates. The drive motor is then stopped, and the light transmission increases with time at a rate proportional to the rate of RBC aggregation. The increase in light transmission during 20 s of blood stasis is measured and displayed as an aggregation index.

The deformability of single RBCs was observed and measured at 22°C using a counter-rotating, cone-plate rheoscope [22] (Effenberger Munich, Germany), which was mounted on an inverted microscope (Leitz Diavert, Wetzlar, Germany). Six shear stresses from 6 to 85 dyn/cm ^{2} were applied, and microphotographs of the cells were taken at each of the shear stresses. Deformation results in elongation of the RBC, and deformation (D) is defined as $0 = (L-W)/(L + W)$, where L is the length and W the width of the deformed cell. To achieve shear stresses causing marked RBC deformation, 5 μl of blood were diluted 1:50 in a dextran solution with a viscosity of 21 mPas (centipoise).

Statistical evaluation

A paired t-test was used to analyze differences between values before and after IL administration within each group. The magnitude of changes produced by IL in the different groups was compared using an unpaired t-test. A value of $P < 0.05$ was considered significant. Data are presented as mean ± standard error of the mean (SEM) unless otherwise specified.

Ethics

The studies were conducted according to the Helsinki Declaration and approved by the Ethical Committee of the Department of Pediatrics of the University of Heidelberg. The parents of all infants and children gave their informed consent.

Results

Several differences in hemorheological parameters before IL infusion were noted among preterm infants, fullterm neonates, and children: blood viscosity, plasma viscosity, and RBC aggregation were lower in preterm infants than in fullterm neonates and reached the highest values in children (Figs. 1 and 2 and Table 1). As previously reported, these observations can be related to differences in total plasma protein and fibrinogen concentrations [20, 21, 23]. The deformability of RBCs studied by means of a rheoscope did not differ among the various groups (Fig. 1).

The results of the measured parameters before and after IL infusion are given in Table 1 and Fig. 1. Basal serum triglyceride levels were lower in neonates than in children. After IL infusion, the highest rise in triglyceride levels was observed in preterm infants. Hemoglobin concentration and hematocrit decreased by about 7%, blood viscosity fell by about 10%, and RBC aggregation fell by 20% (Fig. 1). No significant changes were noted in total plasma protein, plasma albumin, immunoglobulin G, fibrinogen, MCV, MCH, MCHC, plasma viscosity, RBC deformation, or blood viscosity at constant hematocrit of 0.45 (Table 1 and Fig. 1). IL infusion significantly elevated blood pressure in fullterm neonates and children (from 542 ± 7 to 592 ± 8 mmHg in fullterm neonates, $P < 0.05$, and from 732 ± 10 to 782 ± 11 mmHg in children, $P < 0.05$). A slight rise in blood pressure of preterm infants was not shown to be significant (Fig. 2). Although reduction of peripheral blood flow was not significant in any of the three groups, a significant elevation of peripheral resistance (from 4.72 ± 0.6 to 5.22 ± 0.7 mmHg/ml/min 100 ml in preterm infants, $P < 0.05$, from 6.62 ± 0.9 to 7.22 ± 1.2 mmHg/ml/min 100 ml in fullterm neonates, $P < 0.05$ and from 13.82 ± 1.9 to 15.22 ± 2.4 mmHg/ml/min 100 ml in fullterm neonates, $P < 0.05$) and of vascular hindrance, calculated as the ratio of resistance to blood viscosity (R/BV) (from 2.22 ± 0.3 to 2.82 ± 0.5 R/BV in preterm infants, $P < 0.05$, from 2.2 ± 0.4 to 2.92 ± 0.6 R/BV in fullterm neonates, $P < 0.05$ and from 5.42 ± 1.0 to 6.32 ± 0.9 R/BV in children, $P < 0.05$) was found.

Discussion

In agreement with previous studies, we observed that whole blood viscosity at given hematocrit, plasma viscosity, and RBC aggregation were lower in preterm infants than in fullterm neonates and lower in fullterm neonates than in children and adults. These differences have been related to lower plasma proteins in neonates compared with adults [20, 21, 23, 24]. Plasma viscosity is strongly dependent on total plasma protein concentration, but it is more influenced by macroproteins as fibrinogen than by smaller proteins as albumin. Aggregation of RBCs is entirely dependent on the concentra-
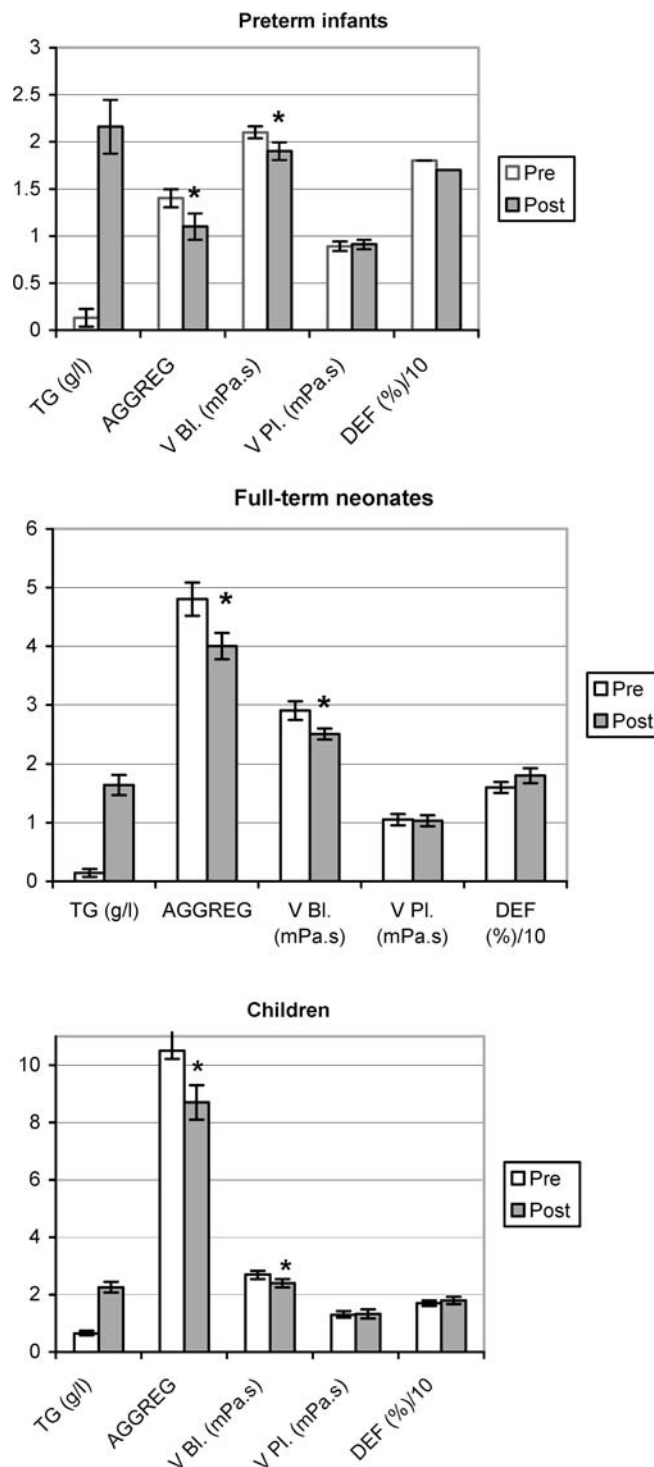

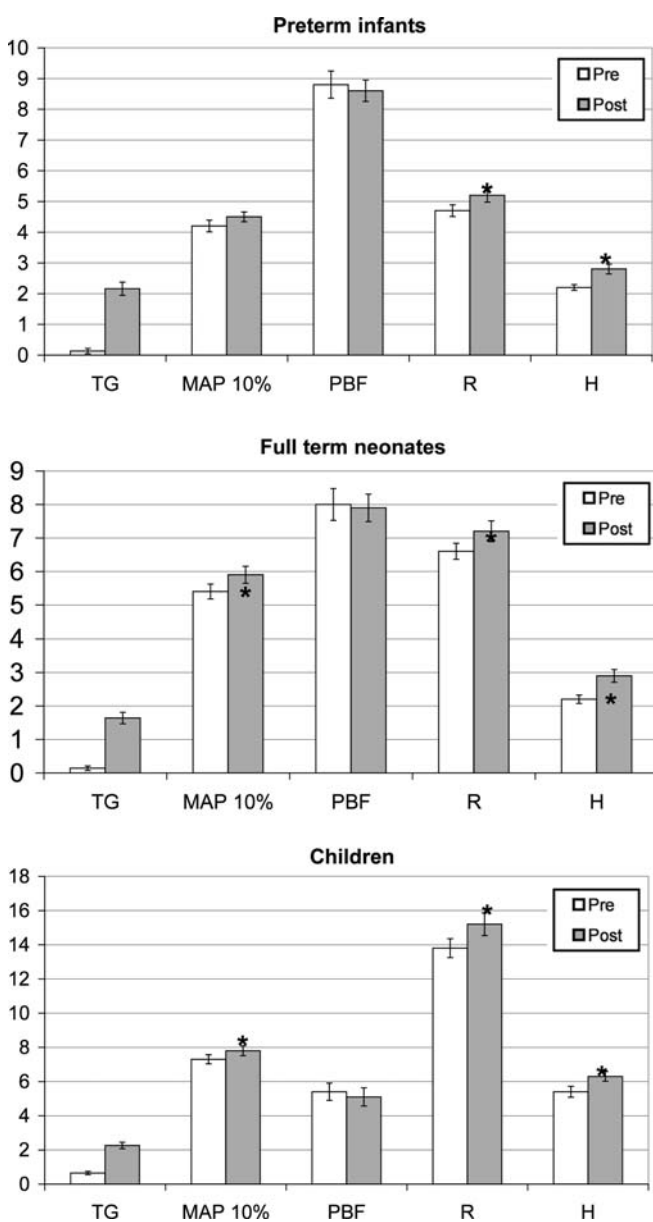**Fig. 1** Effects of Intralipid infusion (0.6 g/kg over 4 h) on hemorheologic parameters

tion of macroproteins forming bridges among adjacent RBC [21, 24]. Thus, the steady increase of macroproteins with gestational and postnatal age explains the concomitant rise in RBC aggregation.

We found that IL infusion caused a marked decrease in RBC aggregation in children and neonates, whereas

Table 1 Effects of Intralipid infusion (0.6 g/kg over 4 h) on hematologic parameters

	Preterm infants		Fullterm neonates		Children	
	Pre	Post	Pre	Post	Pre	Post
Plasma triglycerides (g/l)	0.13 ± 0.09	2.16 ± 0.22	0.14 ± 0.07	1.64 ± 0.17	0.65 ± 0.1	2.26 ± 0.19
Hemoglobin (g/dl)	15.3 ± 0.5	14.2 ± 0.5	16.4 ± 0.6	$14.8 \pm 0.4^*$	12.5 ± 0.3	11.8 ± 0.25
Hematocrit (%)	0.46 ± 0.02	0.43 ± 0.02	50 ± 0.02	$0.46 \pm 0.02^*$	0.39 ± 0.01	$0.37 \pm .01$
MCV (fl)	120 ± 2.8	118 ± 2.5	108 ± 1.9	106 ± 2.2	91 ± 1.3	90 ± 1.3
MCH (pg)	39 ± 1.0	38 ± 1.0	36 ± 0.6	35 ± 1.0	29 ± 0.6	29 ± 1.0
MCHC (g/dl)	33 ± 0.63	33 ± 0.63	33 ± 0.63	32 ± 0.32	32 ± 0.32	32 ± 0.63
Total plasma protein (g/l)	48 ± 1.3	46 ± 1.0	53 ± 1.9	50 ± 0.6	72 ± 2.5	70 ± 2.2
Plasma fibrinogen (g/l)	2.2 ± 0.2	2.1 ± 0.2	2.6 ± 0.2	2.4 ± 0.2	2.8 ± 0.2	2.6 ± 0.2

Values are means $\pm$ SEM; $^*P < O.05$ when compared with basal values (paired t-test)

Fig. 2 Effects of Intralipid infusion for 3 h on circulatory parameters

plasma viscosity and RBC deformation remained unchanged (Fig. 1). As we reported before, the decrease in RBC aggregation following in vitro incubation was strongly dependent on the IL concentration [25]. The in vitro addition of IL decreased plasma proteins by 1% (1 mg/ml) to 8% (8 mg/ml). This decline in macroproteins could only partially explain the marked decrease of RBC aggregation. We have previously shown that a decrease in fibrinogen concentration of 8% reduces RBC aggregation by 22% in a fibrinogen range of 2–3 g/l [21], whereas the decrease in RBC aggregation after the addition of IL at concentrations of 8 mg/ml decreased RBC aggregation in adults by 50% and abolished RBC aggregation in the neonates.

Effects of IL infusion on various rheological properties of blood in adults have recently been reported by Linde et al. [16]. Thirteen healthy adults received a bolus injection of 0.5 ml/kg of IL 20% in 10 min followed by an IL infusion of 90 ml/h over 4 h, resulting in a total IL dose of approximately 0.4 g/kg.

Using a rotational viscometer, Linde et al. estimated RBC aggregation from measurements of whole blood viscosity at a low shear rate of 1/s and RBC deformability from viscosity measurements of RBCs suspended in buffer solution at a hematocrit of 55% and a shear rate of 1/s [16]. They found a decrease in whole blood viscosity of 5%, indicating a decrease in RBC aggregation, and a decrease in the viscosity of RBC buffer suspension of about 10% after IL infusion, suggesting an improvement of RBC deformability. In principle, the viscosity of RBCs suspended in a protein-free medium at given hematocrit is determined by RBC deformability only [22, 26]. However, Linde et al. found no change in whole blood viscosity at a high shear rate of 1/100 s in hematocrit and plasma viscosity after IL infusion, although at constant hematocrit and plasma viscosity, increased RBC deformability should have decreased whole blood viscosity.

In contrast to the report of Linde et al. [15] and our results, Rim et al. found a significant positive correlation between whole blood viscosity (measured in a rotational viscometer at a shear rate of 7.34 S-1) and plasma triglyceride levels in dogs with hyperglyceridemia induced

by IL infusion. It is unclear which of the determinants of blood viscosity (hematocrit, plasma viscosity, RBC deformability, or aggregation) was responsible for the triglyceride-dependent rise in blood viscosity, as only blood viscosity was reported.

The decrease in RBC aggregation after IL infusion may be explained by an increase in the RBC surface negativity [27], thereby increasing the repelling forces between RBCs. Moreover, fatty acids may compete with the binding sites of macroproteins on the RBC surface [28]. Our finding of normalization of RBC aggregation after resuspending RBCs in IL-free plasma indicates that IL decreased RBC aggregation as a result of direct effects of IL compounds on RBCs [25].

Effects of lipids on RBC deformability depend on the composition of the fatty acids. Docosahexaenoic acid has been shown to increase RBC deformability [29, 30]. IL contains little docosahexaenoic acid (0.25%) and therefore does not increase RBC docosahexaenoic acid [30]. This may explain why IL had no effect on RBC deformability.

This is, to our knowledge, the first investigation to demonstrate elevated vasoconstriction after IL infusion in newborns. Vasoconstriction after IL infusion is known to occur in adults, mainly via an endothelium-dependent, still unclear mechanism [31]. Above that, blocking of endothelium-dependent vasorelaxation in adults has been shown to exclusively occur in the presence of heparin [31]. Our observations demonstrate that in newborns and children, mechanisms of vasoconstriction and dilation are different from those in adults.

We conclude that an initial infusion of IL at a dose of 0.6 g/kg given in 4 h does not impair flow properties of blood, but it enhances peripheral vasoconstriction in neonates and children. RBC aggregation appears to decrease during IL infusion, suggesting improved blood flow properties at low shear forces (i.e., in veins). Previously described pulmonary side effects of IL as increasing pulmonary arterial pressure thus cannot be related to impaired flow properties of blood.

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