Group B Streptococcus impairs erythrocyte deformability in neonates more than in adults

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Abstract

Group B β -haemolytic Streptococcus (GBS) may cause severe septic shock and death in neonates, whereas this is rarely the case in adults. As impaired red blood cell (RBC) deformability might disturb microcirculation in septic shock, the in vitro effects of GBS (1.7×10^8 cfu/ml) on RBC deformation (rheoscope) and haemolysis were studied in blood from preterm infants, term neonates, and adults. Furthermore, RBC deformation was studied in term neonates with GBS sepsis. RBC deformation at a shear stress of 4 Pa decreased significantly within 5 minutes of GBS incubation in preterm infants (-13%) and term neonates (-9%). In adults RBC deformation did not change during the first 15 minutes, but decreased significantly after 30 (-10%) and 60 minutes (-13%). In the term infants there was little further decrease in RBC deformation between 5 and 60 minutes of GBS incubation; RBC deformation in preterm infants decreased by 19% after 60 minutes compared with the preincubation values. RBC deformation in septic neonates was significantly decreased at shear stresses of 1, 2, and 3 Pa (-19%, -18%, and -9%). Sixty minutes of incubation of RBC from adults and neonates with GBS and without GBS resulted in haemolysis below 4%.

It is concluded that neither neonatal nor adult RBC are haemolysed by GBS. In vitro, neonatal RBC deformability is more impaired than that in adults. This may contribute to the high risk of neonates for compromised microcirculation and circulatory shock as a result of GBS sepsis.

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Group B Streptococcus (GBS) is a major pathogen in neonatal pneumonia and sepsis. Neonatal GBS infection is frequently associated with increased pulmonary and systemic vascular resistance and decreased cardiac output.¹ The circulatory effects of GBS are similar to those seen in Gram negative septicaemia² and are generally explained by the release and action of various mediators and cytokines such as tumour necrosis factor, interleukins-1 and -6,³ leukotrienes, prostaglandins and thromboxane.⁴ Moreover, hydroxyl radicals may have a role in the cardiopulmonary consequences of GBS septicaemia in neonates.⁵ In neonates disturbed

skin microcirculation is an early sign of sepsis.⁶ Previous studies have shown that impaired circulation in neonates,⁷ children,⁸ and adults⁹ with Gram negative septicaemia is associated with decreased red blood cell (RBC) deformability and increased haemolysis. Moreover, endotoxin severely impairs RBC deformability in vitro.⁷⁻¹⁰ As far as is known, no report on the effect of GBS on RBC deformability has been published before.

This study was designed to investigate RBC deformation and haemolysis in adults, preterm, and term neonates, and umbilical cord blood during in vitro incubation with GBS. Furthermore, RBC deformation was studied in neonates with GBS sepsis.

Methods

A total of 36 newborn infants were studied with the approval of the Ethical Committee of the Children's Hospital of the University of Heidelberg and with the informed consent of the parents. Five groups were studied. Group 1 comprised 10 healthy term neonates with a gestational age of 38-40 weeks. Group 2 included 10 healthy preterm infants with a gestational age of 28-30 weeks. Group 3 comprised 10 umbilical cord blood samples taken from healthy term neonates. Group 4 included 10 healthy adults. RBC in term and preterm neonates (groups 1 and 2) were collected on the first day of birth. Six septic term neonates (group 5) were studied between days 3 and 6 after birth. These infants had a positive blood culture for GBS, raised C-reactive protein (3-11 mg/dl), but no coagulation abnormalities. All of them survived after treatment with ampicillin and tobramycin.

Blood was anticoagulated with heparin (5 IU/ml) and all measurements were done within 4 hours of blood collection. RBC were isolated by centrifugation for 10 minutes, and plasma, buffy coat, and RBC were separated with gentle aspiration. The RBC were washed twice with isotonic phosphate buffered saline solution (pH 7.4, 300 mosmol/kg).

GBS type III/R was isolated from the blood of a newborn infant who had developed early onset sepsis. Bacteria were grown in Todd-Hewitt broth for 24 hours at 37°C and harvested by centrifugation at 5000 rpm for 15 minutes. Bacteria were resuspended in sterile saline 0.9% to a concentration (determined by serial viable counts) of 1×10^9 cfu/ml. The GBS were stored in Todd-Hewitt broth with 25% ethylenglycol and frozen at -18°C. The GBS concentration was determined before each RBC incubation by

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Fig 1A

Fig 1B

Figure 1 Deformation of RBC in the rheoscope. The cells were suspended in isotonic dextran solution and subjected to a shear stress of 1 Pa. (A) Red blood cells from a healthy term infant without GBS incubation; (B) RBC from the same neonate incubated for 30 minutes with GBS (1.7×10^8 cfu/ml) show substantial impairment of RBC elongation.

optical density measurements compared with McFarland (III) solution at 578 nm. For haemolysis and RBC deformation measurements, 500 μ l GBS suspension was incubated with 60 μ l packed RBC and 2440 μ l PBS (GBS: 1.7×10^8 cfu/ml). These suspensions were incubated at 37°C for up to 60 minutes.

Haemolysis was studied before and after 15, 30, and 60 minutes of incubation at 37°C. Haemoglobin was measured at 546 nm in supernatant fluid after 5 minutes of centrifugation at $2000 \times g$. Haemolytic activity was expressed as the ratio haemolysis to maximal haemolysis of the suspension. Maximal haemolysis was achieved by lysis of 60 μ l RBC in 2940 μ l of distilled water.

For the rheoscope deformation measurements, 5 μ l of RBC were diluted 1 in 40 (haematocrit about 0.025 l/l) in PBS containing 20 g/dl of dextran T-2000 (Sigma Chemical, Munich, Germany). The osmolality of this dextran solution was 300 ± 10 mosmol/kg and the viscosity was 21 mPa second. The deformation of a single RBC was observed and measured by a counter-rotating cone plate rheoscope¹¹ (Effenberger, Munich, Germany), mounted on an inverted microscope (Diavert, Leitz, Wetzlar, Germany). Details of this method have been described elsewhere.¹² Four shear stresses of 1, 2, 3, and 4 Pa were applied at a temperature of 23°C and the elongation of RBC was video recorded. Length (L) and width (W) of 30 RBC were measured in each sample using a computed micrometer system (Optimas, Stemmer, Puchheim, Germany). The first 30 completely visible RBC were evaluated. The RBC deformation parameter D

is defined as $D = 100 \times (L - W) / (L + W)$. D increases with increasing cell elongation with a maximum value of 100%.

Results are expressed as mean (SD). Analysis of variance for paired observations was used to test for changes in RBC deformation during 60 minutes of GBS incubation. The three neonatal groups and the adults were compared using analysis of variance for unpaired observations. A P value of <0.05 was regarded as significant.

Results

Figure 1 shows deformation of untreated RBC and of RBC incubated in vitro with GBS (1.7×10^8 cfu/ml) in the rheoscope at a shear stress of 4 Pa. In adults RBC deformation decreased significantly after 30 minutes (-10%) and 60 minutes (-13%) of in vitro incubation with GBS (fig 2). In term neonates, RBC deformation had already decreased significantly after 5 minutes of GBS incubation (-9%) with little further increase during the following 55 minutes (-12%). In the preterm infants RBC deformation was significantly decreased after 5 (-13%) to 60 minutes (-19%) of GBS incubation. Sixty minutes of incubation of RBC from adults and neonates with and without GBS resulted in haemolysis below 3.5%.

Figure 3 shows that GBS caused a significant reduction in RBC deformation after 30 minutes of in vitro GBS incubation at a shear stress of 1, 2, 3, and 4 Pa. RBC in preterm infants had a more pronounced reduction of deformation than adult RBC at each of the four shear stresses. At the highest shear

Figure 2 Effect of group B *Streptococcus* (1.7×10^8 cfu/ml) on RBC deformation (shear stress of 4 Pa) during 60 minutes of incubation. Mean (SD); $P < 0.05$ compared with control values.

stress of 4 Pa, RBC deformation was more reduced in venous and cord blood of term neonates (45.5 (3.1)% and 46.0 (2.9)%, respectively) and in the venous blood of preterm infants (44.2 (3.1)%) than in adults (48.3 (3.1)%).

In septic neonates (fig 4) RBC deformation was significantly decreased (−19%, −18%, and −9%) at shear stresses of 1, 2, and 3 Pa when compared with healthy term neonates without GBS incubation.

Discussion

In vitro incubation of RBC with GBS induced a substantial reduction in RBC deformation at defined shear forces. Impairment of RBC deformation occurred within 5 minutes in neonates and was more pronounced in preterm infants and term neonates than in adults (fig 2). Neonatal and adult RBC showed little haemolysis after 60 minutes of incubation with or

Figure 3 RBC cell deformation after 30 minutes of incubation with GBS (1.7×10^8 cfu/ml) related to shear stresses of 1–4 Pa. Mean (SD); $*P < 0.05$ compared with control values.

Figure 4 RBC deformation in neonates with GBS sepsis ($n=6$) and in healthy neonates ($n=10$). Mean (SD); $*P < 0.05$ compared with healthy neonates.

without GBS. Marchlewicz *et al* isolated a haemolysin of GBS and found that this was dependent on GBS-haemolysin concentration, suggesting that a 'multihit' response is required for RBC lysis.¹³ They found haemolytic activity after washing GBS in PBS, but not in the supernatant fluid of unwashed GBS cultures. This agrees with our results. Impairment of RBC deformability without haemolysis has also been shown for endotoxin.^{10 14}

It is unclear which GBS toxin is responsible for the decrease in RBC deformability and why neonatal (especially preterm) RBC are more affected by GBS than adult RBC. Rojas *et al* found similar effects for live bacteria and their exotoxins on the vascular and pulmonary circulation of sheep.¹⁵ Infusion of GBS exotoxin in sheep provoked strong inflammatory responses in the lung and extensive capillary endothelial damage. Hellerqvist *et al* observed that a GBS polysaccharide exotoxin binds only to developing or immature endothelium in neonates or in tumours, and propose that binding to specific cellular components in immature tissues may be the cause for the high susceptibility of neonates to GBS sepsis.¹⁶ Pauly *et al* propose that hydroxyl radicals may be involved in the cardiopulmonary consequences of GBS septicaemia in neonates.⁵ Because neonatal cells (including RBC) are highly susceptible to peroxidation,¹⁷ more pronounced GBS impairment of neonatal RBC deformation may be caused by increased membrane lipid peroxidation.

We conclude that neither neonatal nor adult RBC are haemolysed by intact GBS type III/R, but that neonatal and adult RBC deformability are significantly decreased by GBS in vitro and in vivo. As the GBS impairment of RBC deformation was more pronounced in preterm infants than in term neonates and least pronounced in adults, we speculate that either GBS toxins binds more readily to neonatal than to adult RBC, or that GBS toxins affect neonatal RBC more than adult RBC. The stronger impairment of neonatal RBC deformation may contribute to the high risk of neonates for GBS induced septic shock.

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