Complement and contact activation in term neonates after fetal acidosis

Josef Sonntag, Mathias H Wagner, Evelyn Strauss, Michael Obladen

Abstract

***Aims*—To evaluate complement and contact activation after fetal acidosis.**

***Methods*—Fifteen term neonates with hypoxic–ischaemic encephalopathy after umbilical arterial pH < 7.10 were compared with 15 healthy neonates with umbilical arterial pH > 7.20. Determinations of the complement function and C1-inhibitor activity were performed as kinetic tests 22–28 hours after birth. C1q, C1-inhibitor, and factor B concentrations were determined by radial immunodiffusion and those of C3a, C5a, and factor XIIa by enzyme immunoabsorbent assay.**

***Results*—Median complement function (46 *vs* 73 %), C1q (4.3 *vs* 9.1 mg/dl), and factor B (5.2 *vs* 7.7 mg/dl) decreased after fetal acidosis. The activated split products C3a (260 *vs* 185 µg/l), C5a (5.0 *vs* 0.6 µg/l), and factor XIIa (3.2 *vs* 1.3 µg/l) increased in the neonates after fetal acidosis. No differences were found in the concentration and activity of C1-inhibitor.**

***Conclusions*—Complement and contact activation occurred in the newborns with hypoxic–ischaemic encephalopathy. Activation of these systems generates mediators which can trigger inflammation and tissue injury.**

(*Arch Dis Child Fetal Neonatal Ed* 1998;**78**:F125–F128)

Keywords: birth asphyxia; complement activation; contact activation; fetal acidosis

The complement and contact systems are activated after ischaemia and reperfusion injury, for example, myocardial infarction,[1 2] cardiac surgery with cardiopulmonary bypass,[3 4] or liver transplantation.[5 6] Fetal acidosis, as a marker for uteroplacental insufficiency, is associated with hypoxia and reperfusion injury in neonates.[7] Complement and contact components in these babies have not been systematically investigated.

This study aimed to assess complement and contact system activation in term neonates with hypoxic–ischaemic encephalopathy after fetal acidosis by examining several complement components and factor XIIa (Hageman factor) (figs 1 and 2).

Methods

The study was approved by the local ethics committee and written parental consent obtained. Inclusion criteria for the study group were: an umbilical arterial pH < 7.10 and hypoxic–ischaemic encephalopathy 20 hours after birth.[8] Grade of hypoxic–ischaemic encephalopathy, renal function (diuresis by weighing the nappies and serum creatinine 24 hours after birth), coagulation disorders (prolonged clinical bleeding time, decrease in platelets, evidence of fibrin split products) and duration of mechanical ventilation were recorded. Seventeen neonates, admitted to our neonatal intensive care unit between July 1995 and March 1996, fulfilled the inclusion criteria. Because two sets of parents did not give their consent, the study involved 15 infants with a median gestational age of 39 weeks (range 37–41). None of patients had evidence of infection (C-reactive protein > 0.5 mg/dl 24 hours after birth or positive blood culture). Fifteen healthy term neonates without perinatal complications, with the same gestational age, and an umbilical arterial pH > 7.20 were enrolled in the control group at the same time.

The study group were monitored to maintain mean arterial blood pressure, body temperature, normoglycaemia, normocalcaemia, normoxaemia, and normocapnia. Therapeutic interventions included infusion of fluids, mechanical ventilation, and administration of glucose and calcium. Five infants with convulsions were treated with phenobarbital, three received erythrocyte transfusion because of anaemia within the first 24 hours. Eleven infants were given pasteurised plasma solutions (Biseko, Biotest, Dreieich, Germany) for volume expansion. None of the patients received dexamethasone.

Blood samples were taken 22–28 hours after birth. Samples of blood (0.4 ml) were collected in two tubes containing either disodium–ethylene diamine tetra acetic acid (EDTA; Kabi-Labortechnik, Germany) or 0.07 ml sodium citrate (Fa; Saarstedt, Germany) and within 20 minutes centrifuged for 5 minutes at 3000 rpm. The plasma was immediately

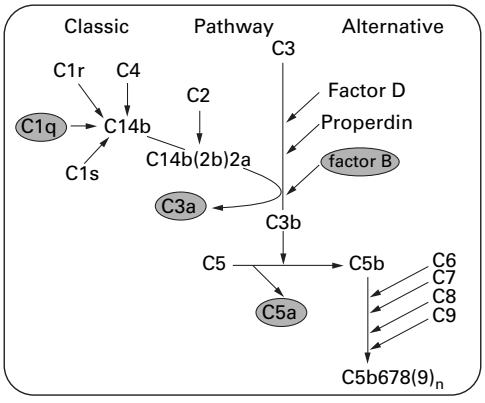

Figure 1 Complement system activation.

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Accepted 18 September

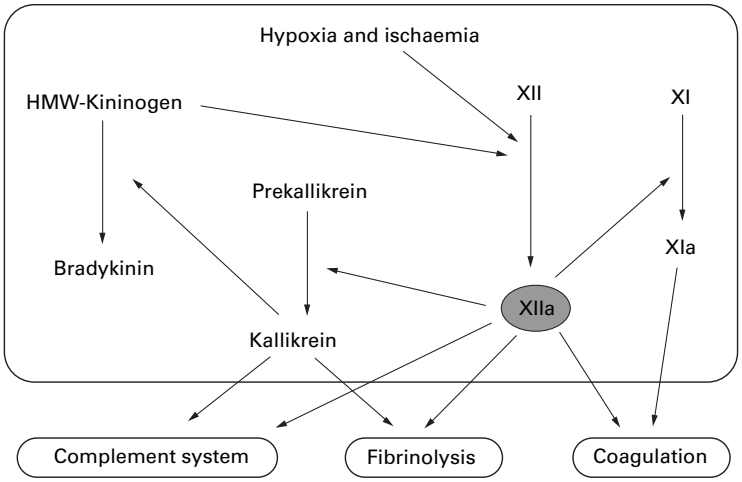

Figure 2 Contact system activation and its influence on complement system.

separated and stored at −80°C for not longer than one month.

The reliability of the test results was routinely monitored using controls with known concentrations or activities. The bioassays were tested without prior knowledge of group assignment or clinical data.

Kinetic determination of the functional whole complement activity is based on the lysis of sensitised sheep erythrocytes (Behring Diagnostica AG, Marburg, Germany) by the activated complement factors in the plasma sample. The test measures the time needed for a total lysis of a fixed number of erythrocytes, which is indicated photometrically at 578 nm.[9] An intra-assay precision study (n = 20) showed a coefficient of variation of 5% using a standard plasma with 50% complement function.

Concentrations of C1-inhibitor, C1 q, and factor B were determined with single radial immunodiffusion using the Nor-Partigen kit (Behring Diagnostica AG, Marburg, Germany).[10] The immunodiffusion diameter was corrected using a standard curve in a semilogarithmic plot. In intra-assay precision studies coefficients of variation (n = 20) were: 4% for a concentration of 5 mg/dl for C1q; 3% for 8.0 mg/dl for factor B; and 6% for 0.10 g/l for C1-inhibitor. The concentrations used to determine the coefficients of variation were within the normal range for neonatal plasma.

C3a enzyme immunoassay (EIA, Fa. Progen Biotechnik GmbH, Heidelberg, Germany) selectively detects C3a-desArg using monoclonal antibodies.[11] The variation coefficient (n = 20) for a concentration of 550 µg/l was 8% for this method. C5a was determined using a specific sandwich EIA (Fa. Behring, Marburg, Germany) and showed a variation coefficient (n = 20) of 8% for a concentration of 5 µg/l.[12]

The concentration of activated factor XIIa was measured using a semiquantitative direct immunoassay with specific sheep monoclonal antibodies (WAK-Chemie Medical GmbH, Bad Homburg, Germany).[13] The intra-assay coefficient of variation (n = 20) for a concentration of 10 µg/l was 6%.

The functional activity of the C1-inhibitor was determined in citrated plasma by using the chromogenic substrate technique described by Heber *et al* (Behring Diagnostica AG, Marburg, Germany).[14] The coefficient of variation in an intra-assay precision study for a standard plasma with an activity of 95% was 5%.

As most of the data were not distributed normally, results were expressed as medians with quartiles. Differences between the two groups were assessed using the Mann-Whitney U test. Significance was assumed at $p < 0.05$. All calculations and tests were carried out using the software package SPSS-PC (Chicago, Illinois USA).

Table 1 Clinical data of acidosis and control group (median with quartiles)

	Acidosios group (n=15)	*Control group (n=15)*	*p Value*
Gestational age (weeks)	39 (38/40)	39 (38/40)	NS
Birthweight (g)	3470 (3180/3655)	3560 (2850/3820)	NS
Umbilical arterial pH	6.96 (6.90/7.05)	7.32 (7.29/7.36)	< 0.0001
Apgar 1 minute	2 (1/3)	9 (9/9)	< 0.0001
Apgar 5 minutes	5 (2/7)	10 (10/10)	< 0.0001
Apgar 10 minutes	7 (4/8)	10 (10/10)	< 0.0001

Table 2 Comparison of concentration and activities of complement factors and Hageman factor between acidosis and control groups (median with quartiles)

	Acidosios group (n=15)	*Control group (n=15)*	*p Value*
Complement function test (%)	46 (24/52)	73 (60/90)	0.001
C1q concentration (mg/dl)	4.3 (3.5/5.7)	9.1 (8.2/9.7)	< 0.0001
Factor B (mg/dl)	5.2 (4.3/7.9)	7.7 (6.9/9.3)	0.02
C3a (µg/l)	260 (180/705)	185 (100/260)	0.04
C5a (µg/l)	5.0 (0.6/10.5)	0.6 (0.55/1.2)	0.005
Factor XIIa (µg/l)	3.2 (1.3/6.4)	1.3 (0.8/1.9)	0.02
C1 inhibitor concentration (g/l)	0.12 (0.08/0.15)	0.12 (0.10/0.16)	NS
C1 inhibitor activity (%)	65 (55/103)	75 (55/110)	NS

Results

Clinical data from the study and control group are shown in table 1. Ten neonates from the study group developed grade I hypoxic–ischaemic encephalopathy, three grade II, and two grade III. Five neonates from the study group had renal failure in the first 48 hours, characterised by diuresis <1.0 ml/kg/hour and serum creatinine >120 µmol/l. Four developed a coagulation disorder in the first three days of life. Nine neonates were intubated at birth and artificially ventilated for 0.5 to 168 hours, two of them developed clinical and radiological signs of surfactant deficiency. None of the infants died before being discharged from hospital. None of the infants from the control group had clinical symptoms of hypoxic–ischaemic encephalopathy, respiratory distress, coagulation disorders, or renal failure.

The study group showed decreased functional activity of the whole complement system as well as decreased plasma concentrations of C1q and factor B. Higher amounts of activated split products C3a, C5a and factor XIIa were found in this group than in the control group. No difference in concentration or functional activity of C1-inhibitor was found between the study and control groups (table 2).

Discussion

Brain damage after severe fetal acidosis may affect the entire life of the child. Many studies have investigated the pathophysiology of the developing tissue injury based on hypoxia and ischaemia and its therapeutical prevention in affected neonates. Aprimary problem is to

detect those babies likely to be at risk. Low umbilical arterial pH and Apgar score are the traditionally used definitions of fetal acidosis, but they are poor predictors of latent disability in surviving infants.[15 16] The best predictors of death or handicap are neurological symptoms in the early neonatal period referred to as hypoxic–ischaemic encephalopathy.[17 18] Therefore, in this study we investigated only those neonates with both fetal acidosis and development of hypoxic–ischaemic encephalopathy within 20 hours of birth.

In the study group concentrations of C1q and factor B in plasma as well as haemolytic activity of the complement system were lower than in the control group. The reasons for the diminished values may be the consumption of the native complement proteins following complement activation, or reduced protein synthesis due to transient liver failure or a combination of both. To verify the complement activation we determined the activated split products acting as anaphylatoxins. C3a and C5a both increased after fetal acidosis. Schrod *et al*[19] reported increased C3a concentrations in preterm neonates with adult respiratory distress syndrome due to the surfactant inactivation after shock. We found increased C3a concentrations in acidotic neonates with (n=2) and without (n=13) respiratory distress syndrome. The increased C5a after fetal acidosis indicates an enhanced production, because C5a first binds to granulocyte receptors and only the free anaphylatoxin molecules were found in plasma.[4] Considering the increased anaphylatoxin concentrations, as well as decreased function and concentration of native proteins, we assumed that the complement system is activated after birth acidosis. Because the complement activation was evident 22 to 28 hours after birth, the effect of therapeutic interventions on the complement system activation within this time frame cannot be excluded. However, we found no differences between the infants who were treated with phenobarbital, transfusion, or pasteurised plasma solutions and those who were not in the study group. None of the study infants received drugs or acute interventions that are reported to influence the immune system. Thus the main reason for the complement activation is probably cell disintegration. Ischaemia releases subcellular constituents—mostly mitochondrial proteins—which bind to C1q and activate the complement cascade in vitro and in vivo.[20 21] Another reason for complement activation is the loss of protective membrane proteins on injured cells, which may be due to the activation of complement cascade in the ischaemic area.[22 23]

The complement system has a major role in initiating some of the inflammatory events occurring in ischaemia and reperfusion after myocardial infarction, cardiopulmonary bypass surgery, and liver transplantation.[2–4 6 24] Anaphylatoxins contribute to an increased permeability of small blood vessels, the contraction of smooth muscles, the release of histamine, the secretion of lysosomal enzymes and cytokines as well as granulocyte migration and adherence. Another aspect of the complement activation is the direct cytotoxic effect of the membrane attack complex on endothelial cells.[2] All these mechanisms may enhance tissue injury following ischaemia and reperfusion.[22 25 26]

The increased concentrations of factor XIIa in the study group may be explained by contact activation after fetal acidosis. The reason for this activation likely is the contact of factor XII with negatively charged surfaces or cell constituents after cell destruction. Additionally, the contact system is activated by hypoxanthine,[27] which increases after birth asphyxia.[28] The activated contact or kinin system is involved in inflammatory tissue injury through bradykinin and kallikrein release with increased vascular permeability, leucocyte accumulation, and arterial hypotension. The contact system is closely related to the complement system, and mutual activation is possible.[27] Additionally, factor XIIa influences coagulation and fibrinolysis,[27] which act simultaneously after birth asphyxia.[29] Thus increased values of factor XIIa can contribute to the development of disseminated intravascular coagulation disorders in acidotic neonates.

Activation of the complement and contact system is controlled by rapid binding of C1-inhibitor to factor C1 and factor XIIa.[30] We therefore expected decreased concentration and activity in the study group after complement and contact activation. But the values were no different from those of the control group despite complement activation. This may be due to increased synthesis of this acute phase protein after asphyxia.[30] However, the additional amount of C1-inhibitor produced could not prevent the activation of both systems.

Complement and contact activation occur in neonates with hypoxic–ischaemic encephalopathy after fetal acidosis. Whether such activation causes tissue damage is not proved by our data, but it generates mediators which can promote inflammation and may contribute to the pathogenesis of reperfusion injury.

This work was supported by the German Research Society (DFG-Ob 43/6-2).

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