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In-vitro activation of complement system by lactic acidosis in newborn and adults

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Introduction: Complement activation occurs secondary to a variety of external stimuli. Lactic acidosis has been previously shown to activate the complement factors C3a and C5a. In the present investigation we examined the differential effect of lactic acidosis on anaphylatoxin levels in cord and adult blood. Furthermore we aimed to determine if the entire complement cascade could be activated by lactic acidosis.
Methods: **Cord and adult blood samples (n = 20 each) were collected and incubated for one hour in either untreated condition or with the addition of lactate in two concentrations (5.5 mmol/l *vs* 22 mmol/l). Following incubation, levels of C3a, C5a and sC5b–9, and blood gas parameters were determined.**
Results: **Anaphylatoxin (C3a and C5a) and sC5b–9 levels increased with the addition of lactate in a dose-dependent manner in cord and adult blood (C3a: 1 h, 5.5 mmol/l, 22 mmol/l: 418/498/622 µg/l in cord blood; 1010/1056/1381 µg/l in adult blood, p<0,05; similar results were found for C5a and sC5b–9).**
Conclusion: **Lactic acidosis leads to an activation of the entire complement system in neonates and in adults. This activation is dose-dependent and more pronounced in adults as compared to neonates.**

Key words: Lactate, Cord blood, Adult blood, Complement, Activation, Anaphylatoxins

Introduction

Activation of the complement system occurs due to a variety of conditions. These are either associated with hypoxia or with cellular damage resulting in acidosis during conditions like birth asphyxia or infection.[1–4] The components of damaged tissue like mitochondrial fractions or other subcellular components cause activation of complement via the alternative pathway.[5,6] As we have shown previously, lactic acidosis itself can result in activation of C5a even in the absence of cellular components.[7] The aim of this study was to investigate whether lactic acidosis led to an activation of the entire complement cascade and to look for differences in complement activation between neonatal and adult blood. Furthermore we aimed to determine whether anticoagulation with heparin or citrate led to different levels of activation of the complement system.

Materials and methods

Following informed consent, blood samples of 10 ml were collected in polypropylene tubes from the placenta of 20 term newborns without acidosis (base excess<–10) and 20 healthy adult volunteers. Each specimen was devided into two samples of 5 ml and either 100 units of heparin or 0.5 ml sodium-citrate was added for anticoagulation. Four 5 ml samples (heparin-placenta-blood, citrate-placenta-blood, heparin-adult-blood, citrate-adult-blood) were devided in 3 portions and remained untreated, were treated with lactate 0.5 mg (5.5 µmol/l) or with lactate 2 mg (22 µmol/ml). Samples were incubated for 60 min at 37°C. Following incubation 1 mg disodium-ethylen-diaminetetra-acetic-acid was added to the heparin samples to stop complement activation and samples were centrifuged immediately. The remainder was stored at –80°C. Blood pH, potassium, lactate concentration, pCO_2, and base excess were measured in all samples prior to and following incubation. C5a and C3a were quantified by the use of a specific sandwich enzyme immuno assay (Behring, Marburg, Germany). The terminal complement complex was measured by the Elisa technique detecting the soluble sC5b–9 (LD Labordiagnostik, Heiden, Germany).

Statistical analysis

As most data were not normally distributed, results were expressed as medians with quartiles. Differences between placental and adult samples were assessed by the Wilcoxon test. Statistical significance was assumed at p<0.05. All calculations and tests were performed with the SPSS-PC-Software 9.0 (Chicago, IL, USA).

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Table 1. Base deficit and lactate concentration for cord and adult blood at 0 h, 1 h, and after equilibration with lactate (5.5 mmol/l and 22 mmol/l)

	Cord blood			Adult blood			Significance
	median	P25	P75	median	P25	P75	
Base deficit							
0 h	8.35	7.03	9.35	3.70	2.30	4.65	$p<0.05$
1 h	10.10	9.00	11.10	4.75	3.85	6.20	$p<0.05$
5.5 mmol/l	15.80	13.83	17.05	10.85	8.43	11.88	$p<0.05$
22 mmol/l	29.25	26.60	31.42	25.25	22.82	27.35	$p<0.05$
Lactate [mmol/l]							
0 h	3.30	2.40	3.88	1.02	0.82	1.18	$p<0.05$
1 h	5.12	4.36	5.51	2.70	2.34	3.23	$p<0.05$
5.5 mmol/l	10.53	8.66	11.68	7.80	7.26	8.95	$p<0.05$
22 mmol/l	21.22	14.45	23.97	18.21	13.75	24.56	n.s.

Results

Baseline levels of pH were not different between umbilical cord (7.34 [7.30/7.38]) and adult venous blood (7.36 [7.35/7.37]). Base deficit (BE) and lactate were significantly higher in neonatal blood (for details see Table 1).

Incubation induced significant changes of pH, base deficit and lactate in both placental and adult blood samples (pH: cord blood: 7.34 at 0 h to 7.28 [7.24/7.31] at 1 h, $p<0.05$; adult blood: 7.36 to 7.34 [7.32/7.36], $p<0.05$; for details of base deficit and lactate see Table 1). Lactate concentrations were higher in placental blood during all conditions when compared to adult blood (for details see Table 1). Levels of potassium were higher in cord blood (data not shown) as was the base deficit (Table 1). Anaphylatoxin (C3a and C5a) and sC5b–9 levels increased from baseline incubation with more pronounced acidosis and showed substantial differences between placental blood and adult blood: complement factors were significantly higher in adult blood (Table 2).

Anticoagulation with citrate rather than heparin did not affect levels of lactate nor base deficit (data not shown). The use of citrate decreased baseline levels of pH (cord blood: 7.29 [7.25/7.34] *vs* 7.34, $p<0.05$; adult blood: 7.32 [7.31/7.34] *vs* 7.36, $p<0.05$). Whereas C3a levels were higher in adult samples when treated with heparin (C3a: 1010 µg/l [882/1148; heparin] *vs* 704 µg/l [292/978; citrate], $p<0.05$), no significant differences existed in cord blood samples. Levels of C5a were different in cord blood samples (C5a: 0.23 µg/l [0.119/0.401; heparin] *vs* 0.646 µg/l [0.314/1.077; citrate], $p<0.05$), but did not differ in adult blood specimens (for details see Table 3).

Discussion

In the present investigation we provide evidence of activation of the entire complement cascade by exposure to lactic acid. Baseline complement levels were lower in neonatal as compared to adult blood, activation of complement occurred in a dose-dependent manner and was less pronounced in neonatal blood.

Table 2. C3a, C5a and sC5b–9 in untreated cord and adult blood at 1 h and after equilibration with lactate (5.5 mmol/l and 22 mmol/l)

	Cord blood			Adult blood			Significance
	median	P25	P75	median	P25	P75	
C3a [µg/l]							
1 h	418	182	560	1010	882	1148	$p<0,05$
5.5 mmol/l	498	240	640	1056	865	1343	$p<0,05$
22 mmol/l	622	243	922	1381	1033	1604	$p<0,05$
C5a [µg/l]							
1 h	0.230	0.119	0.401	0.969	0.605	1.199	$p<0,05$
5.5 mmol/l	0.331	0.116	0.566	1.123	0.232	2.609	$p<0,05$
22 mmol/l	0.650	0.753	1.870	2.458	1.437	4.236	$p<0,05$
SC5b–9 [µg/l]							
1 h	110	72	173	270	220	358	$p<0,05$
5.5 mmol/l	289	217	380	437	366	556	$p<0,05$
22 mmol/l	406	274	590	589	492	717	$p<0,05$

Table 3. Comparison between heparin- and citrate-treated cord and adult blood samples following incubation with and without lactate addition for the parameters C3a, C5a, sC5b–9, and pH

		Cord blood							Adult blood						
		Heparin median	P25	P75	Citrate median	P25	P75	*p*	Heparin median	P25	P75	Citrate median	P25	P75	*p*
C3a															
[μg/l]	1 h	418	182	560	452	187	803	n.s.	1010	882	1148	704	292	978	$p<0.05$
	5.5 mmol/l lactate	498	240	640	581	203	810	n.s.	1056	865	1343	746	362	952	$p<0.05$
C5a															
[μg/l]	1 h	0.23	0.119	0.401	0.646	0.314	1.077	$p<0.05$	0.969	0.605	1.199	1.187	0.595	1.519	n.s.
	5.5 mmol/l lactate	0.331	0.116	0.566	0.984	0.348	1.260	$p<0.05$	1.123	0.232	2.609	1.308	0.707	1.716	n.s.
sC5b–9															
[μg/l]	1 h	110	72	173	136	76	161	n.s.	270	220	358	139	108	211	$p<0.05$
	5.5 mmol/l lactate	289	217	380	181	145	250	$p<0.05$	437	366	556	211	150	280	$p<0.05$
pH															
	1 h	7.28	7.24	7.31	7.24	7.21	7.28	$p<0.05$	7.34	7.32	7.36	7.32	7.30	7.34	n.s.
	5.5 mmol/l lactate	7.10	7.04	7.17	7.07	7.02	7.09	$p<0.05$	7.17	7.12	7.18	7.15	7.13	7.18	n.s.

We have previously shown that lactic acidosis activates complement and contact system in blood of healthy adult volunteers.[8] Furthermore we established reference values for healthy term newborns in umbilical cord blood.[9] In subsequent investigations we could demonstrate that acidosis itself rather than lactate triggered the activation of the complement components C3 and C5 in healthy adult volunteers.[7] We now provide evidence that complement activation in neonatal blood is less readily achieved and results in lower levels as compared to those in adult blood. Furthermore we show that lactic acidosis initiates the entire cascade of complement factors in neonatal and adult blood.

Lactate, which is the final product of the anaerobic metabolism, reflects the degree of hypoxia in peripheral tissues. Early hyperlactatemia in critically ill children has been shown to be associated with a high mortality.[10] During septic shock in adults the prediction of multiple organ failure is feasible on the basis of serial blood lactate determinations.[11] Serial measurements of lactate in ventilated neonates revealed an association between raised lactate levels and mortality.[12] In addition there is evidence that neonatal base deficit or pH cannot be used as a proxy measure of serum lactate due to higher levels of base deficit and lower pH levels in newborn blood.[12] These results confirm our findings of higher levels of lactate and base deficit and a lower pH in cord blood as compared to adult blood.

Complement activation has been demonstrated in a variety of neonatal conditions such as early onset infection,[13] respiratory distress syndrome[14] and perinatal asphyxia.[15] Elevation of serum lactate is a common event during the course of these neonatal diseases. The fact that lactic acidosis leads to complement activation in neonatal blood has not been demonstrated previously, comparable data only exist for adult blood.[7] In addition we have shown that the complement cascade is activated right through to sC5b-9, which indicates that lactic acidosis is capable of initiating an inflammatory response via activation of complement. Coactivation of complement factors and proinflammatory cytokines like IL-6 has been shown by other investigators during cardiopulmonary bypass.[16] Similar data exist for IL-1, tumor necrosis factor (TNF-α), oxygen-derived free radical generation, and complement activation during extracorporeal membrane oxygenation.[17] Whether these activated mediators or their target cells ultimately lead to cellular damage depends on various factors including the energy status of the cells exposed. A rodent model of CNS trauma demonstrated only recently that inflammatory processes alone are able to initiate a cascade of secondary tissue damage, which resulted in progressive cavitation and glial scarring in the CNS.[18] Potential mediators of the associated inflammatory activation were fibrinogen, factor X, and complement protein iC3b. Taken together, there is evidence for a co-activation of several inflammatory mediators by the initial event of lactic acidosis. Activation of this inflammatory cascade may ultimately lead to tissue damage, which may or may not be organ-specific. Clearly further research is required in order to clarify mechanisms of activation and thereby point out potential therapeutic interventions.

Anticoagulation of blood drawn for complement studies is usually performed with heparin due to the interference of EDTA and citrate-related calcium binding with complement assays.[19] Results of a recent investigation suggest that citrate inhibits complement activation in a dose-dependent manner, which implies that citrate levels below a certain threshold may not interfere with the complement system.[20] In our investigation anticoagulation with citrate did not influence levels of complement in a uniform and reproducible manner. Until the interference of citrate levels with the quantification of complement levels is further clarified, we cannot recommend the use of citrate for these investigations.

Conclusion

Lactic acidosis triggers activation of the entire complement cascade in neonatal as well as in adult blood, although less so in neonatal blood. Anaphylatoxin concentrations (C3a, C5a) and levels of the complement activation product sC5b-9 increase during incubation in a time- and dose-dependent manner with more pronounced lactic acidosis. Anticoagulation with citrate results in unpredictible modulations of complement factors and should be avoided for laboratory evaluation of the complement status.

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