Mediators of Inflammation, 7, 49–51 (1998)

In vitro activation of complement and contact system by lactic acidosis

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THE activation of complement and contact systems occurs in reperfusion injuries with initial tissue hypoxia, and lactic acidosis such as mycardial infarction and birth asphyxia. The aim of our experiment was the formal proof of activation by sole lactic acidosis. Lactic acid was added to blood and plasma samples from 10 healthy volunteers. C5a and factor XIIa were measured by EIA after incubation at 37°C for 1 h. Both concentrations increased ($P < 0.0001$ by Friedman analysis) in blood and plasma samples with increasing amount of added lactic acid. Lactic acidosis can activate C5 from the complement system and factor XII from the contact system directly, even in the absence of cellular components.

Key words: Lactic acidosis, Complement system, Contact system, Hageman factor, C5a

Introduction

Activation of complement and contact system has been described in reperfusion injuries with tissue hypoxia and lactic acidosis such as mycardial infarction, birth asphyxia, and gut ischaemia.[1–4] Contact with membrane or mitochondrial fractions and other subcellular components of destroyed cells as well as reaction to other mediators and receptor changes may cause activation. Complement and contact system participate in the pathogenesis of shock and tissue injury after initial activation.[1,2,4] The aim of this study was to investigate the direct effect of lactic acidosis on the activation of C5 from the complement system and on factor XII from the contact system *in vitro*.[5,6,7]. To distinguish the effect of cellular components on these activations we performed our tests with blood and plasma.

Material and Methods

Twenty ml blood samples from 10 healthy volunteers aged 25–40 years were collected in tubes with 400 I.E. Heparin. Heparin blood was filled into six polypropylene tubes of 1 ml portions. The leftover of 14 ml heparin blood was centrifuged immediately at 4°C for 10 min at 3000 ***g***. The resulting plasma was separated and filled into six tubes of 1 ml portions. Anhydrous lactate (Sigma-Aldrich, Deisenhofen, Germany) dissolved in steril water were added in amounts of 0.2 mg (2.2 µmol), 0.5 mg (5.5 µmol), 1 mg (11.1 µmol), or 2 mg (22.2 µmol) either to blood or plasma sample and one blood and plasma sample was left natively. All 10 portions were incubated in a water bath at 37°C for 1 h. One blood and one plasma sample were centrifuged before incubation and stored at −80°C to be used as start value. After incubation 1 mg disodium-ethylenediaminetetra-acetic acid (EDTA) was added to stop complement activation[5] and the samples were centrifuged, separated and stored. Blood-pH, potassium and lactate concentration were examined in the heparin blood samples before and after lactate addition by Ciba Corning 865 (Fernwald, Germany). Changes in plasma pH-value by added lactic acid were similar as in blood samples and therefore not reported separately.

Reliability of test results was monitored using controls with known concentrations of C5a and factor XIIa. C5a was determined with a specific sandwich EIA[8] (Fa. Behring, Marburg, Germany) and showed a variation coefficient (n = 20) of 8%. The concentration of activated factor XIIa was measured by a semi-quantitative direct immunoassay[9] (WAK-Chemie Medical GmbH, Bad Homburg, Germany), and showed a variation coefficient (n = 20) of 6%. Both EIAs did not depend on pH-value in the sample.

Statistical analysis

As most data were not normally distributed, results were expressed as median with quartiles. Changes with increasing lactic acid concentrations were compared with variance analysis (Friedman test). Differences in C5a and factor XIIa between blood and plasma were assessed by multi variance analysis (MANOVA) for repeated measures. Statistical sig-

0962-9351/98/010049-03 $9.00

Table 1. Results as median with (quartiles) of blood gas analysis, concentrations of potassium, and lactate in heparin blood samples and concentrations of C5a, and factor XIIa in heparin blood and plasma after 1 h incubation on 37°C depending on the added lactate ($n = 10$).

Lactate (mmol/l)	pH	Base deficit (mEq/l)	Potassium (mmol/l)	Measured lactate (mmol/l)	C5a (µg/l) blood	C5a (µg/l) plasma	Factor XIIa (µg/l) blood	Factor XIIa (µg/l) plasma
Start value	7.40 (7.38/7.42)	1.5 (0.5/1.9)	4.1 (4.0/4.2)	1.1 (1.0/1.3)	0.2 (0.1/0.3)	0.2 (0.1/0.3)	2.3 (1.2/3.6)	2.1 (1.2/4.1)
0	7.38 (7.36/7.40)	4.8 (3.4/5.4)	4.2 (4.1/4.2)	1.3 (1.2/1.5)	1.4 (0.8/2.9)	3.0 (1.9/4.0)	2.1 (1.4/4.8)	3.3 (1.1/7.4)
2.2	7.32 (7.30/7.36)	6.2 (5.6/7.0)	4.5 (4.4/4.6)	3.4 (3.2/3.7)	1.6 (0.7/2.2)	3.4 (2.0/4.3)	4.2 (2.6/7.3)	5.4 (1.5/9.6)
5.5	7.19 (7.16/7.23)	10.0 (9.2/10.3)	5.2 (5.0/5.5)	7.4 (7.3/7.6)	2.8 (2.0/4.2)	4.5 (3.1/6.9)	6.0 (3.6/11.4)	6.8 (2.2/13.2)
11.1	6.98 (6.90/7.00)	17.1 (16.2/19.6)	6.4 (6.4/6.8)	13.1 (12.2/13.2)	4.4 (3.2/6.6)	4.8 (2.8/6.3)	8.7 (3.5/10.9)	8.2 (2.8/14.4)
22.2	6.63 (6.62/6.66)	27.6 (25.6/29.2)	7.8 (7.5/8.3)	21.5 (20.0/22.3)	12.0 (7.2/24.2)	7.4 (4.5/10.1)	15.6 (7.6/25.4)	30.0 (20.5/41.4)

nificance was assumed at $P < 0.05$. All calculations were performed by means of the software package SPSS-PC (Chicago, Illinois, USA).

Results

The influence of lactate addition on pH, base deficit, measured lactate, and potassium concentrations in blood and C5a and factor XIIa concentrations in blood and plasma are shown in Table 1. Lactate and potassium concentration as well as base deficit increased with the amount of added lactic acid to the samples ($P < 0.0001$). C5a and factor XIIa concentrations increased ($P < 0.0001$) and differences were found for C5a ($P < 0.001$) and factor XIIa ($P < 0.005$) between blood and plasma samples.

Discussion

The activation of complement and contact system is reported in several conditions associated with tissue acidosis. After birth asphyxia both systems were activated in blood pH-values below 7.10.[3] The lowest value of the pH at inflammatory tissue has been reported to be 5.5–6.5.[10] The diagnostic criteria for lactic acidosis include a pH less than 7.35 and blood lactate concentration greater than 5 mmol/l.[11] Therefore, the described *in vitro* model investigates in clinical relevant ranges.

The elevated C5a and factor XIIa values in our series indicate that lactic acidosis can activate parts of the complement and contact system in plasma and blood. As shown by the similar rise in plasma samples, no cellular interaction or contact with destroyed cells is necessary to initiate the complement and contact cascade in acidosis. The differences between heparin blood and plasma may be due to two factors: (1) binding of C5a and factor XIIa to receptors of blood cells,[12] which results in lower C5a and factor XIIa values in blood compared with plasma by low amount of added lactic acid; and (2) additional activation by contact with destroyed cell membranes and receptor changes in blood, which results in higher C5a and factor XIIa values in blood samples by lower pH values causing cell destruction. This haemolytic effect is documented by raising potassium concentration in the samples.

Fishelson *et al.*[13] reported that at pH 6.4 the generation of the C3 convertase of the alternative pathway is increased in blood samples. The classical pathway and the plasma contact system may be activated by conformational changes of C-reactive protein, which results in binding to negatively charged surfaces in acidosis.[10] However, the C5 convertase, which are instrumental in activating C5 with the release of C5a, need a surface upon which the tri-molecular complement protein complexes can assemble. Therefore, the development of C5a in

plasma samples is difficult to explain without granulocytes. Hammer *et al.*[14] reported a complement activation with cleavage of C5 in serum independent of the classical or alternative pathway by pH 6.4, but without development of C5a. Thus, our results may be show that another proteinase is activated by acidosis in plasma, which then cleaves C5 to C5a without any role of the complement convertase. Alternatively, cells remaining in the plasma might provide surfaces for complement convertase assembly.

Conclusion

Lactic acidosis can activate parts of the complement and contact system directly, even in absence of cellular components. Further studies should be directed to determine the mechanisms for activation of C5 and factor XII by lactic acidosis.

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

Received 19 September 1997;
accepted in revised form 18 October 1997