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ORIGINAL ARTICLE

Effects of desmopressin on platelet function under conditions of hypothermia and acidosis: an in vitro study using multiple electrode aggregometry*

A. A. Hanke,[1] C. Dellweg,[2] P. Kienbaum,[3] C. F. Weber,[4] K. Görlinger[5] and N. Rahe-Meyer[6]

1 Staff Anaesthesiologist, 6 Senior Anaesthesiologist, Department of Anaesthesiology and Intensive Care Medicine, Hannover Medical School, Hannover, Germany
2 Medical Student, 3 Assistant Medical Director, Department of Anaesthesiology, University Hospital Düsseldorf, Düsseldorf, Germany
4 Staff Anaesthesiologist, Department of Anaesthesiology and Intensive Care Medicine, University Hospital Frankfurt, Germany
5 Senior Anaesthesiologist, Department of Anaesthesiology and Intensive Care Medicine, University Hospital Essen, Essen, Germany

Summary

Hypothermia and acidosis lead to an impairment of coagulation. It has been demonstrated that desmopressin improves platelet function under hypothermia. We tested platelet function ex vivo during hypothermia and acidosis. Blood samples were taken from 12 healthy subjects and assigned as follows: normal pH, pH 7.2, and pH 7.0, each with and without incubation with desmopressin. Platelet aggregation was assessed by multiple electrode aggregometry. Baseline was normal pH and 36 °C. The other samples were incubated for 30 min and measured at 32 °C. Acidosis significantly impaired aggregation. Desmopressin significantly increased aggregability during hypothermia and acidosis regardless of pH, but did not return it to normal values at low pH. During acidosis and hypothermia, acidosis should be corrected first; desmopressin can then be administered to improve platelet function as a bridge until normothermia can be achieved.

Correspondence to: Dr med. Alexander Hanke
E-mail: hanke.alexander@mh-hannover.de
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Hypothermia and acidosis are common complications in severely injured patients [1, 2]. The negative impact of these conditions on coagulation resulting in coagulopathy has been well described [3–9].

Desmopressin is a synthetic analogue of vasopressin (1-deamino-8-D-aginine vasopressin, DDAVP). Beside its first indication for treatment of cranial diabetes insipidus, it has become the treatment of choice for von Willebrand disease (Type I) and mild haemophilia A after it was shown that DDAVP leads to increased levels of coagulation factor (F) VIII, von Willebrand factor (vWF), and tissue plasminogen activator [10]. It also has been shown to improve platelet function [11–13] even under anti-platelet drug therapy [14] or after cardiopulmonary bypass [15]. DDAVP has been shown to correct hypothermia-induced impairment of primary haemostasis partially [16]. The effect of DDAVP under hypothermia and acidosis has not been evaluated to date. Thus, we tested the hypothesis that DDAVP is able to improve platelet function under both hypothermia and acidosis.

Methods

Following Institutional Review Board approval, this study was conducted in accordance with the Helsinki Declarations and European Unions Convention on Human Rights and Biomedicine.

Twelve healthy volunteers of Caucasian origin participated in the study after oral and written information and written consent. All volunteers were healthy and with no history of abnormal bleeding nor taking coagulation impairing drugs. Blood was drawn into four 4.5-ml tubes containing 20 $\mu g.ml^{-1}$ recombinant hirudin (Dynabyte, Munich, Germany) from a basilic vein using a 18-G cannula. Seven polypropylene tubes were labelled (Tube 1–7) and the collected hirudinised blood was immediately aliquoted into these tubes in 2.7-ml split samples.

The test group (DDAVP+) was defined as samples treated with DDAVP. These samples were prepared yielding a final concentration of 1 nM representing an approximate plasma concentration after recommended treatment with DDAVP of 0.3 $\mu g.kg^{-1}$ [17]. Samples without treatment with DDAVP were assigned to the control group (DDAVP−). Baseline was defined as a sample without DDAVP treatment measured at normal pH and 37 °C. Sample preparation was conducted according to the protocol displayed in Table 1.

Following 30 min of incubation, multiple electrode platelet aggregometry (MEA, Multiplate®; Dynabyte) was performed after activation with adenosine diphosphate (ADPTest; Dynabyte) to assess platelet function; ADP was chosen with respect to the previously shown possibility of detection of DDAVP effects under hypothermia [16]. The MEA technique has been described previously elsewhere [18, 19], but in brief, MEA utilises single-use test cells with two pairs of sensor wires. Whole blood is filled into these cells. Thrombocytes are non adhesive in the resting state but when activated, stick to the sensor wires, enhancing the electrical impedance between the wires. These changes in impedance are recorded over 6 min and displayed as a typical curve (Fig. 1). The most important parameter is the area under the aggregation curve (AUC) which represents overall platelet function. The tests were conducted according to the manufacturer's advice using commercially available test solutions (Dynabyte); the AUC was determined and resulting data were stored in a computer.

The pH of each assay was assessed by an automated blood gas analyser (ABL FLEX-900 blood gas analyser; Radiometer, Copenhagen, Denmark) at 37 °C.

Wilcoxon signed rank tests were performed for comparison between measurements. The effects of acidosis on treatment groups were analysed by Friedman tests. Data were analysed using GRAPHPAD PRISM (Version 4.02; GraphPad Software Inc., San Diego, CA, USA). Considering a confidence interval of 95% an α error p of < 0.05 was defined as statistically significant.

Results

Samples were obtained from seven males and five females, with a mean (SD) age of 29 (4.2) years. The mean (SD) native pH under saline dilution was 7.35 (0.03). Sample preparation with hydrochloric acid led to a pH of 7.19 (0.03) for mild acidosis testing, and a pH of 7.02 (0.03) for severe acidosis testing, respectively. The MEA results are demonstrated in Fig. 2.

At native pH, hypothermia decreased the AUC insignificantly ($p = 0.092$) while DDAVP increased the AUC significantly compared with baseline ($p = 0.034$). Acidosis significantly impaired the AUC in both groups regardless of treatment (both $p < 0.0001$). Under conditions of hypothermia (32 °C) and acidosis, DDAVP increased the AUC compared with the control group when pH was normal ($p < 0.001$), when pH was slightly reduced ($p < 0.001$) and when pH was severely reduced ($p = 0.002$). However, under hypothermia and acidosis, the increased AUC after DDAVP treatment was still markedly below the normal range.

Table 1 Sample preparation protocol. Addition of demopressin (DDAVP) yields a final concentration of 1 nM representing an approximate plasma concentration after recommended treatment with DDAVP. Sodium chloride 0.9% addition was conducted to compensate for dilutional effects.

Group	Goal pH	DDAVP loading solution; µl	Hydrochloric acid (0.25 $mol.l^{-1}$); µl	Hydrochloric acid (1 $mol.l^{-1}$); µl	Dilution compensation: sodium chloride 0.9%; µl
Baseline	Normal	–	–	–	300
DDAVP+	Normal	260	–	–	40
	7.2	260	40	–	–
	7.0	260	–	40	–
DDAVP–	Normal	–	–	–	300
	7.2	–	40	–	260
	7.0	–	–	40	260

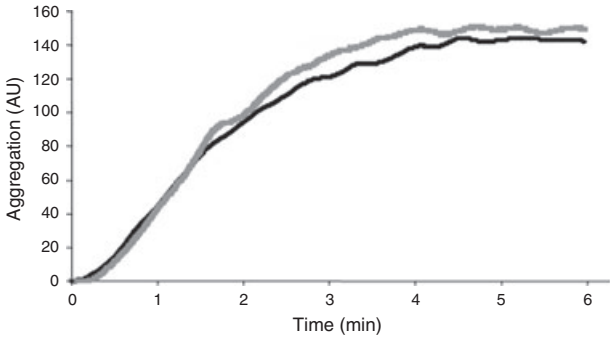

Figure 1 Example of a typical multiple electrode aggregometry tracing (repeated measures of the sample are shown in black and grey). The parameter used is the area under the aggregation curve, which is influenced by the height of the curve as well as by the slope and expresses best overall platelet function.

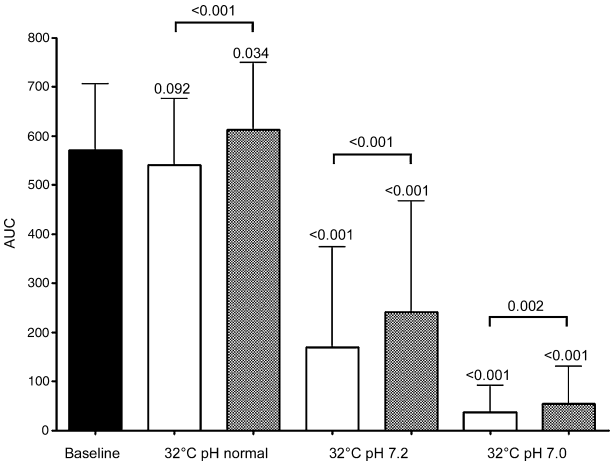

Figure 2 Results of multiple electrode aggregometry after platelet activation by adenosine diphosphate. Results are displayed as mean (SD). Baseline (black bar) was measured at 37 °C without incubation with desmopressin (DDAVP). Groups are DDAVP– (white bars): control group without incubation with DDAVP; and DDAVP+ (grey checked bars): test group with in vitro incubation with DDAVP. p values above the bars show differences compared with baseline, p values above the brackets indicate differences between groups. Note that acidosis severely decreases aggregability and DDAVP leads to an increase of aggregability independent of pH without restoration of aggregability when acidosis is present.

Discussion

Accidental hypothermia, acidosis, and the resulting coagulopathy in trauma patients are clinically known as the so called 'lethal triad of trauma' [1, 20–23]. Mortality in these coagulopathic patients is 13% within the first 24 h and 28% within the hospital stay [24], which is more than 4-fold higher than in patients without coagulopathy [25]. While treatment of acidosis can be enabled easily and rapidly by administration of either sodium bicarbonate or tris buffer, treatment of hypothermia is time consuming. Even by utilisation of the most effective forced airspeed rewarming tools, a warming rate of only 1–2.5 °C per hour is possible [26, 27]. Most recently, case reports have been published indicating a positive in vivo effect of DDAVP in hypothermic trauma patients [28, 29]. Our findings are consistent with their conclusion, implying that in patients suffering from combined acidosis and hypothermia, acidosis should be corrected first and DDAVP might be helpful to increase platelet aggregability for bridging until normothermia can be achieved.

While in our ex vivo study, acidosis severely decreased platelet aggregation, hypothermia alone impaired aggregation insignificantly. We showed that DDAVP increased aggregation irrespective of hypothermia and acidosis in vitro. This improvement of aggregability is most likely to be explained by an increased expression of platelet glycoprotein Ib receptor by redistribution from the cytoplasm to the membrane [30]. However, in the presence of acidosis, platelet aggregation was restored by DDAVP only in part without achieving results within the normal range. One might say that in bleeding patients suffering from severe and treatment resistant acidosis, any improvement of platelet function might be helpful. Whether the statistically significant improvement of aggregability at reduced pH leads to a clinical relevant advantage cannot be assessed by our study. Furthermore, the outcome might be influenced by possible side effects of DDAVP, for example reduction of renal function.

As described before, accidental hypothermia and acidosis are conditions that are consistently found in trauma patients. Since these patients are a very heterogeneous group, we have chosen an in vitro model of hypothermia and acidosis for good comparability of data. Furthermore, coagulation processes are very complex and can be assessed by laboratory tests in part only. Since MEA is carried out in whole blood and is independent of platelet count when severe thrombocytopenia can be excluded [31], we have chosen this particular technique for being as far as possible close to physiological processes. However, we tested the ex vivo effect of DDAVP on platelet aggregation. Whether administration of DDAVP in vivo is also helpful by increasing FVIII and vWF levels under conditions of hypothermia and acidosis cannot be evaluated in our study and should be the subject of further research.

Acknowledgements and competing interests

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