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Influence of UW solution on in vitro platelet aggregability

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Abstract Bleeding problems in orthotopic liver transplantation (OLT), starting immediately after reperfusion of the graft, are complicating the outcome of transplantation. Platelets may be involved in this situation, but there is still a lack of information about the influence of UW solution on platelet function. We evaluated the effect of UW solution on in vitro platelet aggregability in healthy volunteers using whole blood electrical aggregometry and concluded, that UW solution causes impaired platelet aggregability and may contribute to bleeding problems during OLT. The mechanism of impairment remains unclear, since central pathways as well as membrane receptors seem to be involved. Furthermore, our data support the necessity of extended flushing of the liver graft after reperfusion.

Key words Platelets · Whole blood electrical aggregometry · UW solution · Platelet function

Introduction

Bleeding is a significant contributory factor to the postoperative short- and long-term outcome in OLT [1, 3, 17]. Two major causes could be identified to be responsible for this phenomenon. Firstly, hyperfibrinolysis, mainly in the anhepatic phase, and secondly, disseminated intravascular coagulopathy (DIC), starting immediately after reperfusion [3, 4, 6, 13–15]. Another cause may be damage or alteration to the graft liver's vessel wall during the cold ischemic time, suspected because of the beneficial effect of prostaglandin E_1 in the reperfusion injury of the liver [8, 12]. However, other factors may be involved. Some studies have pointed out a decreased platelet aggregability to be one of these factors. Himmelreich et al. found a reduced platelet count and a reduced maximum amplitude of platelet aggregation in platelet rich plasma (PRP) 5 min post reperfusion compared to 5 min before reperfusion. Furthermore, samples taken from the perfusate were significantly lower than systemic samples [7, 9], partly explained by the influence of the UW solution [5].

Whole blood aggregometry (WBEA) was first described in 1980 [2], but did not reach clinical importance because of considerable interindividual differences due to the influences of hematocrit and platelet count. Furthermore, the interpretation of aggregation curves was more intuitive than quantitative. So this convincing method was reserved to a small group of experienced investigators. We established a software package with an A/D converter enabling a mathematical analysis of aggregation curves [16] and a correction for the influence

of hematocrit and platelet count [11]. On the one hand, these improvements pushed the reliability of WBEA to an acceptable standard for a laboratory method, enabling reproducible information on in vitro platelet function, and on the other hand, the advantages of WBEA, quick information and easy handling, were enforced.

The aim of this study was to evaluate the influence of UW solution on in vitro platelet aggregability in whole blood.

Materials and methods

Five female and five male, healthy, non-smoking volunteers (mean age 28.4 ± 3.5 years), who denied intake of any medication throughout the 14 days prior to sampling, participated in this study. Following aseptic venipuncture of the cubital vein, blood was drawn directly into a 5 ml Vacutainer tube containing 0.5 ml of 0.129 M buffered sodium citrate (3.8 %). Samples were measured for red and white blood cells, mean cellular volume, and platelet count using an electronic particle counter (Coulter Counter, T-540). The hematocrit was calculated from the red blood cell count and mean cellular volume. Platelet counts, hematocrit, and white blood cell counts were within normal ranges for all probands.

One milliliter of citrated whole blood was transferred to special polystyrene tubes (Chrono-log Corporation, Haverton, Pa.) containing 0 µl (control), 50 µl (group B), 75 µl (group C), or 100 µl (group D) UW solution (supplied by Beltzer). Thereafter, samples were incubated at 37 °C and stirred at 700 rpm for 10 min. To avoid possible artefacts [10], platelet aggregation testing was performed within 1 h of blood collection using a whole blood aggregometer (Chrono-log Corporation, Haverton, Pa.). Aggregation was triggered using arachidonic acid (AA), adenosine diphosphate (ADP), and collagen (COL), AA at a final concentration of 500 µM, ADP at a final concentration of 10 µM, and COL at a final concentration of 5 µg/ml. [All trigger substances were from Chrono-log Corporation, Haverton, Pa. (Chrono-par)]. The original aggregation data were transferred to a PC/XT-386, the area under the aggregation curve (A-under) was calculated for each sample [16] and corrected for the influence of hematocrit and platelet count [11]. A-under reflects not only the maximum amplitude, but also the steepness of the curve, allowing the quantification of the complete aggregation process. Differences between groups were checked by means of a non-parametric *t*-test (Wilcoxon two-sample test).

Results

Platelet aggregability for each trigger substance, quantified by the parameter A-under, decreased with the addition of 50 µl UW solution. Samples stimulated with ADP showed a significant dose-dependent reduction of platelet aggregability compared to the control group, whereas for samples stimulated with AA or COL, no significant differences were found with higher concentrations of UW solution (75 or 100 µl). Detailed data for each group and trigger substance are given in Table 1.

Table 1 Area under aggregation curve (*AA* arachidonic acid, *ADP* adenosine diphosphate, *COL* collagen)

Trigger substance		Control	Group B	Group C	Group D
AA	Mean	5229	4736	5120	5198
	SD	867	1245	1049	1154
	P value (vs control)		< 0.049	n.s.	n.s.
ADP	Mean	6305	4526	4130	4060
	SD	1267	1209	1066	967
	P value (vs control)		< 0.046	< 0.0051	< 0.0038
COL	Mean	10042	8932	10603	10235
	SD	2341	1072	1856	1788
	P value (vs control)		< 0.036	n.s.	n.s.

Discussion

UW solution is widely used for the preservation of organs during orthotopic liver transplantation, since it allows an extension of the cold storage time with a lower incidence of thrombosis in the hepatic artery and higher survival rates. During liver transplantation, after flushing and reperfusion of the graft, bleeding complicates and influences the short- and long-term outcome of the transplantation procedure. Although other causes of impaired hemostasis have been identified previously, the role of platelets in this context is not well defined. Only one study was found in the literature reporting impaired platelet aggregation in platelet-rich plasma, at least partially caused by UW solution [5]. Therefore, we evaluated the influence of UW solution on in vitro platelet aggregability in ten healthy volunteers using whole blood electrical aggregometry, a method first described in 1980 [2] and modified by our group in recent years [11, 16]. Since testing is performed in citrated whole blood, this method offers a more physiological milieu than platelet aggregation in platelet-rich plasma, which has been used for most studies in this field.

Our results show an impaired AA-, ADP-, and COL-triggered platelet aggregation caused by the addition of 50 µl/ml UW-solution. The stimulation with ADP resulted in a dose-dependent impairment, whereas for aggregation triggered by AA and COL, no significant differences were found with higher concentrations of UW solution (groups C, D). This pattern of aggregation responses is in concurrence with the findings of Himmelreich et al. [5] and implies that UW solution may effect platelet aggregation not only via receptor inhibition, but probably also via a central mechanism. The individual ingredients of UW solution may influence platelet aggregation in one direction (inhibition or stimulation), but the interaction of all component may result in diverse action depending on the concentration. Platelet

aggregation triggered with AA and COL was only reduced at a concentration of 50 µl/ml UW solution. At this concentration, inhibiting effects of UW solution may prevail. However, ADP-triggered aggregation showed a dose-dependent impairment, suggesting an additional competitive mechanism at the membrane receptor. Altogether, the mechanism of impaired platelet aggregation caused by UW solution is still unclear and needs further investigation.

We conclude that UW solution causes impaired platelet aggregation in whole blood, probably contributing to bleeding complications directly after reperfusion. To prevent this side effect, extensive flushing of the reperfused graft is necessary.

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