Heparin-like effect resistant to protamine in a child with haemorrhagic shock. Do we need heparinase?

Mirco Nacoti[1], Diego Cantù[2], Daniele Bonacina[1], Federico Lussana[3], Ezio Bonanomi[1], Marina Marchetti[4], Anna Falanga[4]

[1]Paediatric Intensive Care Unit, "Papa Giovanni XXIII" Hospital, Bergamo; [2]Department of Perioperative Medicine and Intensive Care, "San Gerardo" Hospital, Monza; [3]Haematology and Bone Marrow Transplant Unit, "Papa Giovanni XXIII" Hospital, Bergamo; [4]Division of Immunohaematology and Transfusion Medicine and Haemostasis and Thrombosis Centre, "Papa Giovanni XXIII" Hospital, Bergamo, Italy

Introduction

In patients with chronic and acute liver disease and sepsis, the liver is exposed to a neutrophil-mediated injury and inflammation, which may lead to the release of endogenous heparinoids (e.g. heparan sulphate and dermatan sulphate) from the vascular endothelium into the blood stream[1]. These substances can cause a heparin-like effect (HLE) by inhibiting activated clotting factor Xa in blood and may, therefore, contribute to the coagulopathy and increased risk of bleeding in these patients[2-5]. In this regard, it is well known that the liver contains abundant parenchymal deposits of endogenous heparinoids, heparan sulphate being the predominant[2,3]. Additionally, in patients with acute liver failure, the ability to eliminate circulating HLE substances is likely to be greatly impaired due to the important reduction of liver function. Furthermore, during sepsis, mast cells can release HLE substances[6].

The only drug approved in the USA and European Union to reverse heparin activity is protamine sulphate. This drug has a good binding affinity for high molecular weight molecules, such as unfractionated heparin, but is unable to antagonise low molecular weight heparins[7]. HLE substances typically have a molecular weight similar to that of low molecular weight heparins, thus are not neutralised by protamine. In contrast, heparinase I, an enzyme isolated from Flavobacterium heparinum, is able to neutralise not only unfractionated heparin, but also low molecular weight heparins and several HLE substances[7-9].

Thromboelastography (TEG) is a global haemostatic assay which, upon addition of a variety of activators and inhibitors, is capable of evaluating various parameters of haemostasis simultaneously in whole blood. When conducted in the presence of heparinase I, it can reveal the presence of heparin and HLE[7].

Case report

We describe the case of an 8-month old boy who underwent liver transplantation for biliary atresia at our Institution. The transplant was performed using a split liver (segments III and IV) from a deceased donor, and biliary reconstruction was achieved by a Roux-en-Y hepatic jejunostomy. The liver transplant was complicated by severe intra-operative bleeding and slow recovery of graft function. On day 5 after the transplant, the child was discharged from the surgical ward and transferred to the paediatric department. On day 7, he presented biliary leakage through the abdominal drainage and underwent exploratory surgery: the bowel was not perforated and only liquefied tissue, present near the graft, was found. He was admitted to the paediatric intensive care unit and treated with empirical antibiotic therapy for localised abdominal sepsis without any organ or haemodynamic dysfunction.

After one week, the clinical signs of sepsis and biliary leakage had disappeared.

On the day 15 after the liver transplant, the child developed sudden haemorrhagic shock and metabolic acidosis. Laboratory tests showed a fast clinical evolution towards multiple organ failure. An urgent laparotomy was performed and bowel perforation was found. The liver appeared hypoperfused, and there was an evident haemoperitoneum. While the prothrombin time and activated partial thromboplastin time were only slightly altered, intra-operative bleeding was uncontrolled. A TEG assay was performed and revealed the presence of a severe coagulopathy with no clot formation. TEG showed a straight line for blood with kaolin (no trace) and a reversible configuration after addition of heparinase I to the assay (Figure 1), suggesting the presence of HLE. The definition of HLE varies in the literature and is based on the percentage of correction of the TEG trace by addition of heparinase in vitro. In accordance with the definition of HLE formulated by Senzolo *et al.*[3] we defined HLE as a correction of reaction time (r + k times) on TEG of more than 50% when performing the test in the presence of heparinase. In the absence of any heparin administration, our patient showed a correction of r + k times on TEG of more than 80% with heparinase addition to the assay, suggesting the presence in plasma of HLE substances as a possible contributing factor to the massive bleeding.

Blood Transfus 2018; 16: 394-6 DOI 10.2450/2017.0088-17
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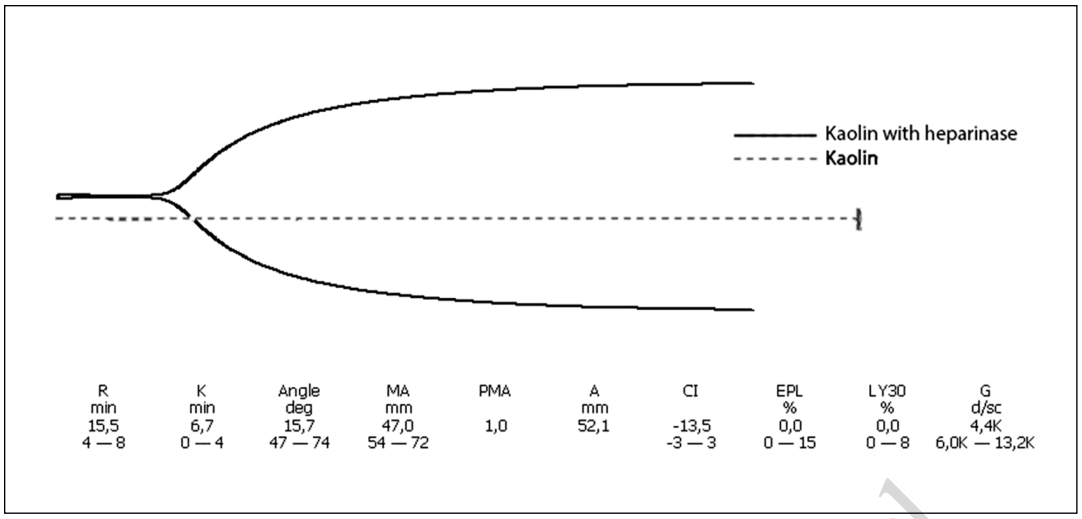


Figure 1 - Thromboelastography at the beginning of surgery.
Continuous trace: test with heparinase I; dotted trace: test without heparinise.
R: reaction time; K: coagulation time; MA: maximun amplitude; PMA: platelet mapping assay; A: alpha angle; CI: coagulation index; EPL: estimated percent lysis; LY30: lysis at 30 minutes; G: clot strenght.

The haemorrhage was continuous and untreatable in spite of massive transfusion of packed red blood cells, fresh-frozen plasma and platelet concentrates. The transfusion protocol for massive bleeding was adopted (packed red blood cells:fresh-frozen plasma = 1:1). No specific areas of bleeding were identified during surgery. TEG was repeated and results were similar: a straight line with kaolin and a partially reversible configuration with heparinase I.

Protamine, as an antidote of heparin, was administrated to the child at a dose of up to 15 mg without any variation on the repeated kaolin TEG result. There was a rationale for using an additional antidote, such as heparinase I, had it been available. In the absence of that, we continued treatment according to the massive transfusion protocol. The child died of untreatable haemorrhagic shock two hours later. The last TEG assay performed still confirmed a partially reversible configuration after addition of heparinase I. No histological abnormalities of the liver were found during the post-mortem examination.

Discussion

In this manuscript, we report a very rare case of untreatable haemorrhagic shock associated with acute liver failure and multiple organ dysfunction, due to abdominal infection, occurring in an 8-month old child, 1 month after liver transplantation for biliary atresia. We observed a severe coagulopathy, as reflected by the straight line on the TEG assay performed with a kaolin-treated sample. The TEG line was improved in vitro by addition of heparinase, suggesting the presence of HLE substances[3,7], probably released in the systemic circulation during sepsis. The administration of protamine to the child did not improve the clinical outcome of severe bleeding nor did it reverse the results of TEG, supporting the hypothesis of the presence of circulating substances not antagonised by protamine as a possible cause of the massive bleeding.

Heparinase I has been used successfully in human studies to reverse the effects of heparin after cardiopulmonary bypass and may be an alternative to protamine[10-13]. However, its role in the treatment of patients with coagulopathy associated with the presence of HLE substances has not been established by extensive clinical studies. Indeed, protamine still remains the only approved drug with anti-heparin activity. The clinical relevance of the presence of HLE in relation to bleeding tendency[3] and the safety profile of heparinase I[13] deserve further exploration.

In this paediatric case report we describe, for the first time, the presence of massive bleeding and HLE, as determined by TEG, in association with sepsis and acute liver failure.

Although further data are necessary, the current evidence supports the hypothesis that heparinase may reverse post-reperfusion coagulopathy during liver transplantation[7] and antagonise the anticoagulant effects of low molecular weight heparins and heparinoids not reversed by protamine[14].

In this small patient protamine was totally ineffective in controlling the severe haemorrhagic coagulopathy. On the basis of the in vitro TEG results, the use of an alternative antidote would have been appropriate.

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Heparinase I has not been approved so far by the Food and Drug Administration for human use. Our clinical case leads us to believe that heparinise could have a role in specific clinical scenarios in the case of inefficacy of protamine and deserves ad hoc investigations.

Authorship contributions

MN and EB designed the report; DC collected the patient's clinical data; MM, DB and FL analysed the data and wrote the paper; AF analysed the data and revised the manuscript.

Institutional review board statement

The Institutional Review Board of "Papa Giovanni XXIII" Hospital does not require ethical approval for case reports.

Keywords: bleeding, children, liver failure, thromboelastography.

The Authors declare no conflicts of interest.

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Arrived: 28 March 2017 - Revision accepted: 19 April 2017
Correspondence: Anna Falanga
Division of Immunohaematology and Transfusion Medicine and Haemostasis and Thrombosis Centre
Ospedale Papa Giovanni XXIII
Piazza OMS 1
24127 Bergamo, Italy
e-mail: annafalanga@yahoo.com

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