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Weibel-Palade Bodies-Presumed Role in Clinical Phase of STEC-HUS

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Abstract

In Thrombotic thrombocytopenic purpura ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) activity is deficient and microangiopathy occurs after a second trigger. In Shiga toxin producing *Escherichia coli* (STEC-HUS) stimulated release of Weibel-Palade bodies (WBP's) is presumed to be the first hit in damaging Gb3 positive endothelial cells in the diarrhea phase. The objective role of the release of WBP's components after admission of the patients is not adequately evaluated. Different mechanisms involved in the pathogenesis are discussed and approaches for treatment proposed. It includes complement system, active coagulation, cytokines/chemokines, cell-free hemoglobin/heme and finally the release of constituents of WPB`s. After admission all evaluated constituents are increased in serum/plasma. Preferred treatment, inhibiting the damaging effect is testing the use of anti-P-selectin antibodies and complete complement inhibition by Pegcetacoplan in order also to prevent long-term sequelae.

Keywords: Weibel-Palade bodies, STEC-HUS, P-selectin, Pegcetacoplan

Introduction

The Weibel-Palade bodies (WPB's) form a unique secretory carrier for Von Willebrand factor (VWF), and other cargo produced by endothelial cells. The main components, although not complete, are presented in **(Table 1)**. These proteins are ready without *de novo* synthesis. Related to their presumed role in the clinical phase of hemolytic uremic syndrome secondary to Shiga toxin-producing *Escherichia coli* (STEC-HUS) selected information is provided. STEC-HUS is characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia and acute kidney injury. Shiga toxin injures endothelial cells during the first few days of infection.

Table 1. Main constituents of Weibel-Palade bodies.

VWF
P-selectin
CD63
Endothelin
Angiopoetin-2

Results

Selected information about components of Weibel-Palade bodies

P-selectin is rapidly exposed on the surface of platelets and on endothelial surface after stimulation. Re-expression of functional characterized P-selectin molecules occurs after repeated stimulation by thrombin. It is recycled [1]. P-selectin binds to leucocyte P-selectin glycoprotein ligand (PSGL1) leading to firm adhesion and transmigration of leucocytes through the vessel wall. It also induces expression of tissue factor on monocytes. CD63 is an essential cofactor for P-selectin function.

Endothelin: ET-1, the major endothelial isoform, is secreted on the abluminal surface of endothelial cells. Plasma concentrations of ET-1 do not accurately reflect ET-1 production.

Angiopoetin-2 (Ang-2): Ang-2 results in destabilization of endothelium, rending it responsive to stimulation by

inflammatory cytokines such as TNF, interleukin-1, and angiogenic cytokines (VEGF).

VWF: From the large number of WPB's undergoing exocytosis upon stimulated, released VWF multimers assemble into VWF strings on the apical side of the endothelium. They have increased platelet adhesive capacity compared with the low molecular VWF. Unfolded VWF multimers can be cleaved by ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13). VWF can recruit leucocytes via direct leucocyte binding or by recruiting platelets, which in turn will attract leucocytes.

Weibel-Palade bodies-function in thrombotic thrombo -cytopenic purpura (TTP) and in diarrhea phase of STEC-HUS

In TTP ADAMTS-13 activity is deficient and systemic aggregation of platelets will occur after a second trigger. In STEC-HUS stimulated release of WPB's components in the diarrhea phase of the disease can be presumed to be the first hit in the damage of Gb3 positive endothelial cells [2]. The role of the release of WPB's content in the clinical phase of STEC-HUS needs further evaluation and will be discussed in the next section.

Weibel-Palade bodies- suggested function in the field of other pathogenic factors in the clinical phase of STEC-HUS

Insight into the pathogenesis of STEC-HUS is provided by Tarr *et al.* [3]. The development of HUS is related to the degree of prothrombotic activation early in infection. Endothelium is a likely target of the absorbed Shiga toxin. A more complete recent view is presented by Michael *et al*. [4]. Targeting by Stx or Stx associated microvesicles to Gb3-expressing endothelial cells, followed by docking and release of toxin by microvesicles is the key pathogenic mechanism proposed. Other mechanisms of cytotoxicity include increased synthesis of chemokines and adhesion molecules, complement activation, elevated free hemoglobin without referring accurately to the possible role of Weibel-Palade bodies. The different mechanisms will be presented in more detail, as accurate as possible, with a proposal to eliminate or reduce the damaging effect. The damaged effect on glomerular endothelial cells has characteristic pathological features. The endothelial cells are swollen and detached from the basement membrane with debris in the subendothelial space. The different mechanisms presented are the complement system, coagulation, anti-Shiga toxin, cytokines/chemokines, heme followed by the role of Weibel-Palade bodies

Complement system

Systemic complement activation of the alternative pathway of complement is observed in the clinical phase of STEC-HUS. Plasma levels of sC5b-9 and Bb are increased [5,6]. A survey of assumed intracellular events is presented by Zoja *et al.* [7]. Eculizumab, a humanized monoclonal antibody binds to C5, preventing its cleavage by C5 convertase and the production of both C5a and C5b-90 complex. Inconsistent results in the treatment of STEC-HUS were obtained. In a recent study eculizumab treatment did not appear to be associated with improved renal outcome during the acute phase of the disease but may reduce long-term-kidney sequelae [8]. It is possible that eculizumab may prevent neurological damage [9]. As C3 is cleaved to generate C3b, a small peptide C3a is released. C3a is increased in plasma in the acute phase of the disease. Pegcetacoplan prevents cleavage of C3 blocking formation of all downstream metabolites, an almost complete complement inhibition [10]. C3a is a small anaphylatoxin mediating pro-inflammatory effects by binding to the G protein-coupled complement receptor C3aR. The complement receptor is widely expressed. C3a is also expressed on endothelial and epithelial cells [11]. Stimulation of C3a on endothelial cells induces a rapid mobilization of WPB's.

Coagulation

In STEC-HUS at admission active coagulation is present (thrombin-antithrombin III complex, prothrombin fragment 1+2 are increased) [12]. Heparin therapy, however, has no benefit [13,14]. Pivotal studies by Weitz *et al.* [15,16] demonstrated that clot-bound thrombin is protected from inhibition by heparin. This occurs in STEC-HUS at admission. Fibrinopeptide-A was increased in 13 out of 16 children. Only in 3 out of 9 children fibrinopeptide-A normalized after heparin administration [17]. Bivalirudin has the ability to inhibit clot-bound thrombin [18] with a possible role in treatment of STEC-HUS. Due to their small molecular weight direct, oral anticoagulants can be considered, although more evidence is required [19].

Anti-Shiga toxin

The study about the effect of equine anti-Shiga toxin is accomplished [20]. These results will indicate a possible beneficial effect.

Cytokines/chemokines

A survey of the increased serum cytokines/chemokines levels observed after admission in STEC-HUS is reported by Shimuzu [21]. It concerns mainly tumor necrosis factor, Interleukin-1β, IL-6, IL-8. Chemokines (MCP, IL-8) activate leucocytes and promote extravasation [22]. Cytokines generate endothelial cell adhesion molecule expression, slowing blood flow and increase vascular permeability [23]. Blocking of these chemokines and cytokines by antibodies is possible.

Heme

Intravascular hemolysis due to mechanical damage to red blood cells by microthrombi result in cell-free hemoglobin and heme, overwhelming endogenous scavengers haptoglobin

and hemopexin. It induces systemic inflammation, vascular dysfunction, thrombophilia and kidney injury as reviewed by Von Avondt *et al.* [24] and Pinto *et al.* [25]. Striking is the exposure of P-selectin on endothelial cells [26,27]. Clinical trials are currently underway for the use of hemopexin and haptoglobin.

Weibel-Palade bodies

A large variety of agonists triggering the exocytosis of Weibel-Palade bodies (WPB's) are well known such as C3a, C5a, heme [2]. A complete exocytosis of all components of the WPB's is not always occurring (**Figure 1**). After stimulation and expression of P-selectin on the cell membrane, functional P-selectin is recycled. Resynthesis is required for other components. Intracellular Ang-2 is detectable 6 hrs after complete release and recovered within 16 hrs.

Figure 1. Confocal microscopy of GMVECs incubated with thrombin or Stx1 in flow conditions. GMVEC donors A and B were incubated in flow with thrombin and Stx1 (1 dyne/cm2). The cells were fixed and stained for VWF (green) and CD31 (red). The merge of fluorescent signals is shown. GMVECs B show less effect of thrombin than GMVECs A. A noticeable effect can be detected in both donors after exposure to Stx1. Permission is granted by S. Karger AG, Basel. Nephron Extra 2014.

After admission of STEC-HUS an increase in serum/plasma of all studied components of WPB's are observed. It concerns Von Willebrand factor (VWF) multimers although with a decreased size [28], P-selectin [29], Ang-2 [30]. Caplacizumab prevents platelet binding of VWF to receptor GPIb-IX-V and the formation of platelet-rich thrombi and is effective in the treatment of TTP. Using new technology ADAMTS-13 activity is only slightly decreased [31]. P-selectin is not only exposed on endothelial cells but also on the surface of activated platelets promoting leucocyte activation. An oral inhibitor famotidine and humanized monoclonal anti-P-selectin antibody (crizanlizumab) are available.

Targeting Ang-2 is an option [32], although it does not directly prove the role of stimulated endothelin secreted on the abluminal surface is expected. ET-1 induces cytoskeletal (actin) remodeling in podocytes and loss of slit-diaphragm proteins such as nephrin. It increases glomerular permeability. Et-1 receptor antagonists inhibit these effects [33].

Our preferred new approaches for treatment are presented in **(Table 2)**.

Table 2. Preferred approaches.
Anti-P-selectin
Pegcetacoplan
Bivalirudin

To elucidate the pathogenesis and possible treatment a plethora of models in small animals, imitating STEC-HUS are explored. None, however, reflects STEC-HUS entirely [34]. In addition, the obtained data cannot predict the effect in humans. Gnotobiotic piglets infected orally with enterohemorrhagic *E. coli* showed thrombotic microangiopathy in the kidneys without, however, hematologic findings [35].

Volume expansion has a mitigating role in STEC-HUS [36]. Other approaches, however, are needed considering the long-term outcome [37]. Fifty-one percent of 281 patients evolved to chronic renal disease (CKD) after 12 years of follow-up and 14% to CKD [2-5].

Conclusion

New approaches for the treatment of STEC-HUS after admission should be evaluated, considering not only the acute mortality but also the long-term prognosis. Targeting WPBs (especially P-selectin) might offer new therapeutic avenue outside the application of pegcetacoplan and bivalirudin.

Conflict of Interest

No conflict of interest.

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