ELSEVIER

Blood 142 (2023) 3975–3977

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

321.COAGULATION AND FIBRINOLYSIS: BASIC AND TRANSLATIONAL

Targeted Inhibition of Phosphodiesterase (PDE) 4 As a Novel Therapy to Increase Endothelial Cell cAMP and Trigger Weibel Palade Body Exocytosis

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Background
Desmopressin (DDAVP) is widely used in the treatment of VWD and other bleeding disorders. DDAVP activates V2 receptors (V2R) on endothelial cells (EC) to stimulate cAMP generation and Weibel-Palade body (WPB) exocytosis. However, DDAVP has inherent limitations, including lack of oral formulation, side-effects related to renal V2R agonism and sub-optimal responses in some patients.

Aims
To identify previously approved drugs with capacity to trigger cAMP-dependent WPB secretion in EC to repurpose as novel hemostatic therapeutic agents.

Methods
Candidate agents were identified by mechanistic screening of FDA and EMA-approved drug databases and rationalized for translational relevance. For each class, prototypical drugs were tested in vitro for capacity to induce VWF release from macrovascular (HUVEC) and microvascular (HMVEC-L) EC (VWF antigen [Ag], propeptide [pp] and collagen binding [CB] in supernatant at 1 hour, expressed as fold increase relative to untreated control [NC]). VWF string formation on the surface of treated EC under flow conditions was determined using fluorescent anti-VWF antibodies and confocal microscopy. Finally, the effects of candidates on platelet aggregation were determined by light transmission aggregometry (LTA).

Results and Discussion
Mechanistic screening and preliminary in vitro evaluation determined a lead candidate drug class: PDE-4 inhibitors. Previous studies have shown that cAMP-hydrolyzing PDE isoforms 2, 3 and 4 are expressed in EC and that non-selective PDE inhibition (PDE-I) with IBMX alone can elevate cAMP in EC sufficiently to induce VWF release. Consistently, we observed that treatment of EC with IBMX significantly enhanced VWF secretion (median fold increase VWF:Ag vs NC:1.49, $p<0.0001$, Fig.1). However, PDE isoforms 2 and 3 are also present in platelets, where increased cAMP activity inhibits platelet aggregation. We confirmed by LTA that IBMX significantly attenuated TRAP-6-induced platelet aggregation, limiting the therapeutic relevance of non-selective PDE-I as VWF-raising agents.
We next evaluated the capacity of isoform-selective PDE-I to induce VWF release. Selective inhibition of PDE-2 (with EHNA) and PDE-3 (with Cilostamide) had no significant effect ($p>0.999$, Fig.1). In contrast, selective PDE-4 inhibition with Roflumilast (ROF) resulted in a dose-dependent increase in VWF:Ag secretion in both HUVEC (1.51 fold, $p<0.0001$, Fig.1) and HMVEC-L (1.75 fold, $p=0.007$). Notably, these effects were also seen with ROF concentrations in the nanomolar range, consistent with plasma levels following single oral dose in healthy subjects.
In keeping with a role for PDE-4 in regulating cAMP-mediated WPB exocytosis, ROF treatment of EC resulted in a proportionate increase in VWFpp secretion (1.74 fold, $p=0.0002$). Furthermore, pre-incubation of HUVEC with ROF triggered VWF string formation under flow conditions, with strings visualized in most fields of view (Fig.2, 80% ROF vs 0% NC, $p=0.0003$). Consistently, following treatment with ROF, increased VWF:Ag was accompanied by a parallel rise in VWF:CB in the supernatant (1.50 fold, $p=0.0061$), confirming the secretion of hemostatically active VWF multimers. Critically, however, ROF had no inhibitory effect on platelet aggregation, consistent with lack of PDE-4 isoform activity in platelets. Finally, ROF combined with either histamine (HIS) or thrombin (THR) had synergistic effects on VWF release, suggesting ROF could prime EC to physiological agonists at times of hemostatic challenge (3.4 fold HIS vs 5.17 fold HIS+ROF, $p<0.0001$; 3.7 fold THR vs 4.3 fold

THR+ROF, p=0.0060). In addition, ROF exhibited synergistic activity with the cAMP-raising agent Isoprenaline compared to ROF alone (1.77 vs 1.40 fold, p=0.0040, Fig.1), suggesting combination therapy as a novel therapeutic approach.

Conclusion

Drug repurposing may allow for rapid and cost-effective translation into clinical practice. Importantly, PDE-4 inhibitors such as ROF are currently licensed oral agents for treating Psoriasis and COPD. Our novel findings identify PDE-4 as a critical endothelial PDE isoform that regulates WPB exocytosis and VWF secretion. Moreover, we demonstrate that ROF stimulates VWF release in vitro without deleterious effects on platelet aggregation. Our data highlight the therapeutic potential of PDE-4 inhibitors, either alone or in combination with DDAVP, in the treatment of VWD.

Disclosures Doherty: *Takeda:* Honoraria; *NovoNorodisk:* Other: Sponsorship (Educational Support); *Amgen:* Other: Sponsorship (Educational Support). **Karampini:** *CSL:* Research Funding. **O'Sullivan:** *Leo Pharma:* Research Funding. **Lavin:** *Takeda:* Honoraria, Other: Indirect funding for development of educational content, Speakers Bureau; *CSL Behring:* Consultancy, Honoraria; *Band Therapeutics:* Consultancy; *Pfizer:* Honoraria; *Sobi:* Consultancy, Honoraria, Speakers Bureau. **O'Donnell:** *Baxter:* Membership on an entity's Board of Directors or advisory committees, Research Funding, Speakers Bureau; *Bayer:* Membership on an entity's Board of Directors or advisory committees, Research Funding, Speakers Bureau; *Novo Norodisk:* Research Funding, Speakers Bureau; *Boehringer Ingelheim:* Membership on an entity's Board of Directors or advisory committees, Speakers Bureau; *Leo Pharma:* Speakers Bureau; *Takeda:* Membership on an entity's Board of Directors or advisory committees, Research Funding, Speakers Bureau; *Octapharma:* Membership on an entity's Board of Directors or advisory committees, Speakers Bureau; *Pfizer:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *CSL Behring:* Membership on an entity's Board of Directors or advisory committees; *Daiichi Sankyo:* Membership on an entity's Board of Directors or advisory committees; *Shire:* Research Funding.

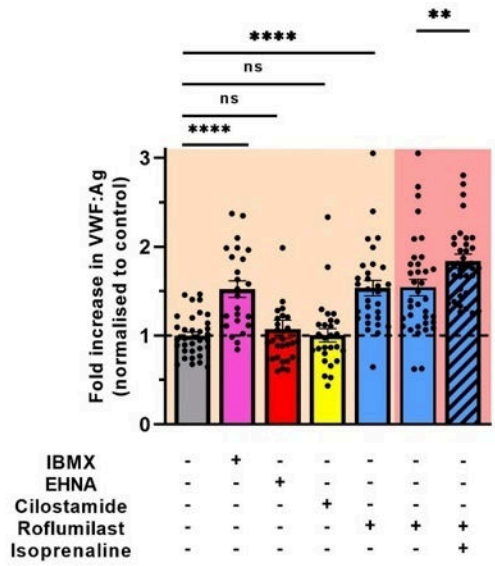

Figure 1. Targeted Inhibition of PDE4 induces VWF secretion. VWF:Ag in supernatant following stimulation of HUVEC for 1 hour with: Vehicle (Negative Control), Non-specific PDE inhibitor (IBMX), PDE-2 inhibitor (EHNA), PDE-3 inhibitor (Cilostamide) or PDE-4 inhibitor (Roflumilast) (left of figure). In addition, Roflumilast alone or in combination with β-Adrenoreceptor agonist (Isoprenaline) is illustrated (right of figure). Data normalised to negative control and visualised as mean +/- SEM. Statistical analyses performed in GraphPad Prism, using Kruskal-Wallis test with Dunn's multiple comparisons test, and Mann-Whitney test. ns=not significant, **=p<0.01, ****=p<0.0001.

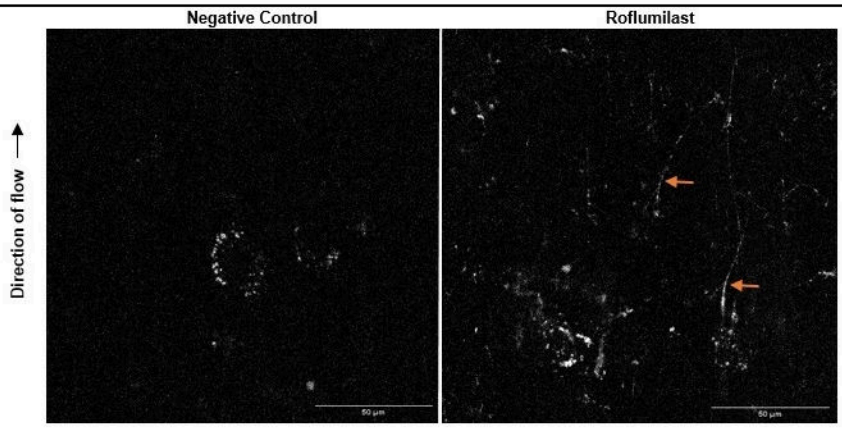

Figure 2. Roflumilast induces secretion of VWF strings under flow conditions. Representative images of VWF string formation on the surface of confluent HUVEC treated with vehicle (Negative Control) or Roflumilast under flow conditions, using fluorescent anti-VWF antibodies and confocal microscopy. Orange arrows highlight the presence of VWF string formation in Roflumilast treated cells. Scale bar represents 50μm.

Figure 1

https://doi.org/10.1182/blood-2023-178581