

# The Inaugural Professor Barry Firkin Oration: “Ristocetin, what have we learnt”

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In the 1970s, the promising and effective vancomycin family antibiotic, ristocetin, from *Nocardia lurida*, was withdrawn from human use because of an unexpected pro-thrombotic effect – an effect not apparent in animal trials. To his everlasting credit, Professor Barry Firkin and his colleagues at Monash University in Melbourne did not simply dismiss ristocetin as a failed, commercially nonviable drug, but investigated the molecular mechanism underlying its pro-thrombotic activity. The consequences of this far-sighted decision have resonated in the haemostasis and thrombosis field for over thirty years. Ristocetin, it was subsequently found, causes platelet aggregation by specifically inducing binding of von Willebrand factor (VWF) in plasma to its receptor, the glycoprotein (GP)Ib-IX-V complex on platelets. The interaction of platelet GPIb-IX-V and VWF multimers in plasma, or associated with the vessel wall (subendothelial matrix or bound to activated endothelial cells), is critical to initiation of platelet aggregate (or thrombus) formation at high shear stress in flowing blood. *In vivo*, this interaction is only triggered by injury exposing subendothelial matrix (activating immobilized VWF) or by pathological shear stress in a stenotic blood vessel (inducing plasma VWF-GPIb-IX-V interaction). Ristocetin-dependent binding of VWF to platelet GPIb-IX-V inducing platelet aggregation under stirring conditions *in vitro* directly mimics this shear-dependent adhesion. It has made a fundamental contribution to understanding haemostasis/thrombosis in three important ways: first, ristocetin-dependent platelet aggregation is a long-standing diagnostic test of patient VWF- and/or GPIb-IX-V-dependent platelet function; second, ristocetin is extensively employed as a research tool for dissecting the molecular interactions involved in ligand-receptor recognition, through analysis of control or mutant patient samples, inhibitory antibodies against VWF or GPIb alpha-chain (the ligand-binding subunit of GPIb-IX-V), or of cross-species GPIb chimaeras; and third, the analysis of the ristocetin-recognition site on the VWF A1 domain led to the identification of a proline-rich sequence (DLAPEAPPPTLPP) flanking A1 as an important regulatory site in activation of VWF. Peptides containing the proline-rich sequence of human VWF bind to ristocetin, although not necessarily at the same site as that recognising the bacterial cell wall D-Ala-D-Ala dipeptide sequence. Notably, the predilection of ristocetin for recognition of VWF in humans, but not other species such as mouse, rat, pig or dog, for example, where the proline-rich sequence is poorly conserved, has provided a unique approach to analysis of human platelet GPIb function. In contrast to ristocetin, other non-physiological modulators of VWF such as the snake toxin, botrocetin (a C-type lectin-family protein) act promiscuously across species *via* a mechanism distinct from that of ristocetin. Thus, comparing ristocetin-dependent *versus* botrocetin-dependent VWF binding to cross-species chimaeras of GPIb expressed on CHO cells has revealed precise functional sites mediating patho/physiological thrombus formation, results confirmed under conditions of hydrodynamic flow. Further, in 2003-2004, the first crystal structures of VWF-A1 in complex with the ligand-binding fragment of GPIb have begun to explain the molecular basis for how this interaction is mediated, and how ristocetin binding to VWF (or GPIb-IX-V) could cause the platelet aggregation that frustrated the early applications of ristocetin as an antibiotic. Together, these diverse studies stemming from the prescient and careful observations of

Professor Firkin have had a profound effect on current understanding of human thrombosis relevant to heart attack and stroke. The original curiosity which inspired them remains a salient lesson for present day investigators in biomedical research.