Thrombosis: Pathology and Therapies

Introduction: haemostasis and thrombosis

Haemostasis is a normal physiological repair process involving the formation of a blood clot to arrest bleeding at the site of vascular injury and preserve the integrity of the vasculature (Kumar et al., 2018). This occurs in response to components in the bloodstream being exposed to underlying collagen fibres in the subendothelial tissue as a result of a compromised endothelial layer. Upon exposure, collagen indirectly tethers platelets to the site of injury via the bound plasma von Willebrand factor (vWF). The interaction between the platelet (GP)Ib-IX-V complex and vWF temporarily immobilises platelets under conditions of high sheer stress, and direct collagen-platelet interaction via integrin $\alpha_2\beta_1$ is necessary to consolidate platelet arrest (Nuyttens et al., 2011). This then allows for the initiation of platelet activation induced by subsequent binding of platelet GPVI receptor to collagen, resulting in a conformational change of the platelet from a resting state to an activated state with increased surface area (Nuyttens et al., 2011; Kumar et al., 2018). In this state, platelets secrete ADP, thromboxane A_2 (TxA_2) and thrombin, which act in an autocrine and paracrine manner to further recruit circulating platelets, amplify platelet activation signals and promote cross-linking of GPIIb/IIIa on platelets with fibrinogen. Aggregation of fibrinogen and platelets progresses, ultimately leading to the formation of a platelet plug which is stabilised by thrombin-regulated conversion of fibrinogen to fibrin (Kumar et al., 2018; Rivera et al., 2009). With haemostasis, the process ends with repair of tissue and reabsorption of the clot, induced by anti-coagulation factors (Kumar et al., 2018).

Despite the haemostatic advantages associated with thrombus formation, abnormal activation of this process can lead to pathological growth of the thrombus (Kumar et al., 2018). This process is known as thrombosis and can occur in intact endothelial tissue (Kumar et al., 2018). Endothelial cells play a crucial role in balancing anti- and pro-thrombotic signals which regulate thrombin production and activity (Kumar et al., 2018). However, these cells are highly sensitive to local changes and prothrombotic gene expression can be induced by many stimuli including inflammation, altered metabolic activity and altered blood flow due to atherosclerosis (Kumar et al., 2018; McFadyen et al., 2018). Diseases such as diabetes mellitus and obesity are therefore able to predispose individuals to thrombosis due to the propagation of a

chronic inflammatory environment and the production of advanced glycation end products which cause oxidative damage and accumulation of cells with the potential to aggregate in the fibrin mesh (Vazzana et al., 2012).

Although many of the mechanisms involved in these two processes cross over, emerging studies have identified differences in the components involved in the haemostatic and thrombotic response (McFadyen et al., 2018). Whilst the initial platelet plug at the core of the thrombus is comprised of highly activated platelets sensitive to signalling by thrombin, ADP and TxA_2, the outer thrombotic layer is composed of platelets at a low activation state regulated by alternative mechanisms (McFadyen et al., 2018). These differences provide a potential platform for the development of novel drugs specific for thrombus growth under pathological conditions (McFadyen et al., 2018).

Pathogenesis of associated diseases

The progression of thrombosis can manifest in many ways, underlying many life-threatening cardiovascular diseases (Kumar et al., 2018). Among non-communicable diseases, cardiovascular conditions are the leading cause of mortality globally, with stroke and ischemic heart disease accounting for approximately 85% of cardiovascular deaths (Naghavi et al., 2017). In Australia, CVD is a major disease burden, contributing to elevated morbidity and mortality rates whilst also indirectly impacting Australia's economic productivity (Marquina et al., 2021).

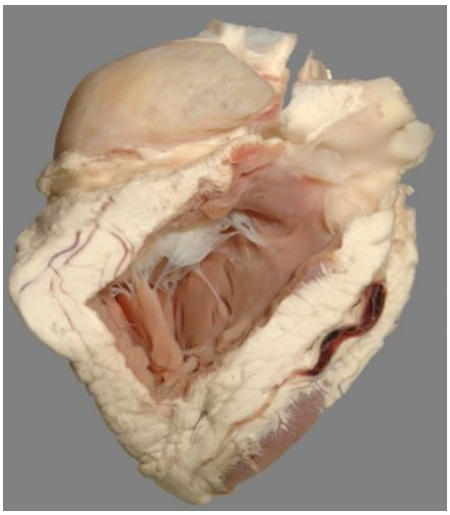

Figure 1. Thrombus in the coronary artery. Abbot No. 11.36.1. Ainsworth Pathology Museum

The pathogenesis and clinical manifestations of thrombi depend largely on the blood pressure conditions and hence the site of thrombus formation (Kumar et al., 2018). Arterial thrombosis often occurs as a result of atherosclerosis due to the rupture of an atherosclerotic plaque or altered blood flow (Turpie & Esmon, 2011). With arterial thrombosis, the major concern is partial or complete obstruction of biologically important arteries by an enlarged thrombus, particularly the coronary or cerebral arteries, leading to ischemia of the respective organs (Kumar et al., 2018).

Obstruction of the coronary vessels, as seen in the branch of the left coronary artery in specimen 11.36.1, typically results in ischemic heart disease, or myocardial infarction in the case of myocardial cell death (Institute of Medicine et al., 2010).

Conversely, in the case of venous thrombosis, stasis due to immobilisation or other factors is usually the cause of thrombus formation which typically occurs in superficial or deep veins of the leg (Turpie & Esmon, 2011). This is termed deep vein thrombosis (DVT) and is seen in specimen 16.344.2 which shows occlusion of the large left femoral vein (right in the image) by a thrombus arising from the left calf. Clinical symptoms include pain felt locally, as well as swelling of the respective leg due to oedema in local tissue (Kumar et al., 2018). Microscopically, organisation of the thrombus has occurred, with new capillary growth throughout the thrombus to restore blood flow in the vessel (Kumar et al., 2018). Due to the restored function of the vein, DVT does not often present with clinical symptoms (Kumar et al., 2018). This poses a problem medically as 50% of patients present as asymptomatic before complications arise, resulting in a sudden and unpredictable death (Kumar et al., 2018; Tapson, 2008).

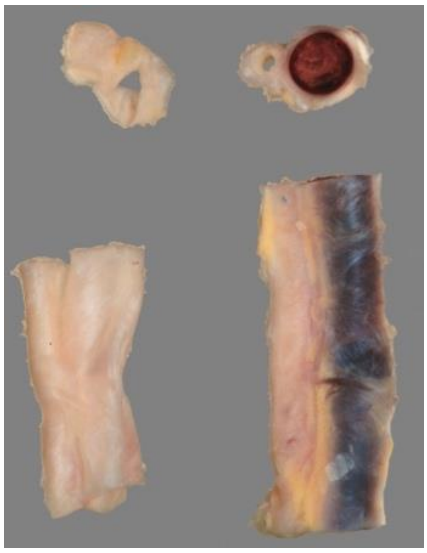

Figure 2. Femoral vein thrombosis. Abbot No. 16.344.2. Ainsworth Pathology Museum.

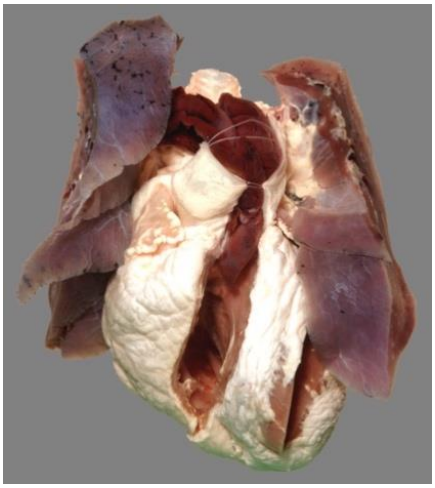

Figure 3. Massive pulmonary embolism. Abbot No. 24.351.2. Ainsworth Pathology Museum.

The major complication of a venous thrombus is the potential for embolisation and deposition in distant vessels (Turpie & Esmon, 2011). Pulmonary embolism is a common outcome of deep vein thrombosis and is the leading cause of cardiovascular-related mortalities after myocardial infarction and stroke (Goldhaber & Bounameaux, 2012). This is displayed in specimen 24.351.2 where there is obvious blockage of the pulmonary trunk and smaller pulmonary arteries.

Current therapies for DVT and pulmonary embolism

Following embolism of a thrombus, patients are typically treated with a course of anticoagulants (Goldhaber & Bounameaux, 2012). Recommended initial treatments involve the use of low-molecular-weight (LMW) heparin in conjunction with long-term treatment with warfarin to reduce the risk of recurrent thromboembolism (Goldhaber & Bounameaux, 2012). This is due to superior efficacy and bleeding profiles compared to other therapies demonstrated in clinical trials (Goldhaber & Bounameaux, 2012; Hovens et al., 2006).

Heparin is a potent anticoagulant which acts on the thrombin pathway. Typically, vascular injury exposes tissue factor to the bloodstream, resulting in downstream reactions which leads to the conversion of prothrombin to thrombin by activated factor X (FXa) (Hemker, 2016). Thrombin is a potent platelet activator and is also able to catalyse the conversion of fibrinogen to fibrin, thus playing a dual role in platelet activation and aggregation (Kumar et al., 2018). To balance thrombin generation, antithrombin III (ATIII) inactivates thrombin as well as FXa at a slow rate (Goldhaber & Bounameaux, 2012). Reversible binding of heparin to ATIII accelerates this process, thus reducing thrombin levels and having an overall antithrombotic effect (Goldhaber & Bounameaux, 2012).

Unfractionated heparin has demonstrated a reduction in 2-week mortality of acute pulmonary embolism patients by 70% (Agnelli & Becattini, 2015). Whilst the efficacy of unfractionated heparin has proven to be higher than many other forms of medication in several trials, the difficulties associated with treatment are problematic (Goldhaber & Bounameaux, 2012). Due to massive differences in the binding of heparin to plasma proteins between individuals, monitoring of the thrombin-pathway through blood tests is needed to inform adjustments of heparin dose (Goldhaber & Bounameaux, 2012). To combat this, LMW heparins were discovered, with the advantage being the use of fixed doses without the need for monitoring (Goldhaber & Bounameaux, 2012). These LMW heparins shared similar levels of recurrent pulmonary thrombosis prevention and similar bleeding profiles to unfractionated heparins (Agnelli & Becattini, 2015). Despite the relatively low risk of bleeding compared to other therapies, all current anticoagulants on the market are associated with an increased bleeding risk, limiting the therapeutic use of these drugs (Goldhaber & Bounameaux, 2012).

Novel antithrombotic therapies

Current antiplatelet and anticoagulant therapies have endured use over many centuries for treatment of thrombosis-related diseases. However, increased levels of resistance in particular cohorts to these therapies necessitates increased dosage or discovery of more potent drugs (White et al., 2020; Spiess, 2008). This is restricted by the inherent bleeding risk that is associated with antithrombotic drugs (McFadyen et al., 2018). However, emerging studies identifying differences in the composition of thrombi and mechanisms involved in haemostatic and thrombotic responses have allowed for the development of novel drugs which theoretically have the potential to target thrombosis while maintaining haemostasis (McFadyen et al., 2018).

One of these novel drug classes which also interferes with the thrombin-mediated pathway are the protease-activated receptor 4 (PAR4) inhibitors (McFadyen et al., 2018). In addition to fibrin generation, thrombin reinforces thrombus formation by amplifying platelet activation via cleavage of the bound ligand of the PARs G-protein receptor (De Candia, 2012). This allows the ligand to bind to the active binding site of the receptor, resulting in downstream signalling and ultimately platelet aggregation (De Candia, 2012). Inhibition of the PARs receptors therefore prevents thrombin-mediated signalling, leading to potent reductions in platelet activation and aggregation (McFadyen et al., 2018). PAR1 antagonists such as vorapaxar have been approved for clinical use (McFadyen et al., 2018). However, due to the critical role that PAR1 plays in early thrombus formation, PAR1 inhibitors pose substantial risks of bleeding and hence its therapeutic use is limited (Wong et al., 2017).

However, studies determining the role of PARs receptors demonstrated significantly slower rates of activation of PAR4 compared to PAR1 as well as activation in higher thrombin concentration conditions due to the lower thrombin-affinity (De Candia, 2012). As a result, inhibition of PAR4 was shown to have minimal impact on bleeding but is hypothesised to be responsible for propagation of the thrombus (De Candia, 2012; Wong et al., 2017). PAR4 antagonists are therefore expected to have improved safety profiles compared to current PAR1 inhibitors and other antithrombotic drugs.

The recent discovery of the PAR4 inhibitor, BMS-986120, following high-throughput screening showed promising results, with pre-clinical monkey studies demonstrating significant reductions in thrombus weight, surpassing that of clopidogrel, a commonly used anti-platelet drug (Wong et al., 2017). The high efficacy and safety profile suggested in the pre-clinical studies have allowed for the progression of BMS-986120 into phase I clinical trials (McFadyen et al., 2018). Despite promising pre-clinical studies, species differences in PARs receptors may pose potential problems for the translation of the results to humans in clinical trials (De Candia, 2012). Different types of PARs receptors were found in other mammalian species, resulting in differences in the extent of thrombosis-modulation (De Candia, 2012). Additionally, PAR4 was suspected to potentially be redundant in humans due to the presence of PAR1, rendering inhibition useless if this proves true (De Candia, 2012).

Conclusion

Thrombosis underpins some of the most serious cardiovascular diseases, contributing to a large proportion of non-communicable morbidities and mortalities in the developing world (Naghavi et al., 2017). Despite current therapies being highly efficacious, the close relationship between hemostasis and thrombosis poses major bleeding risks, limiting the therapeutic window of these agents. However, due to novel discoveries distinguishing hemostasis and thrombosis, many novel therapies have been developed with theoretically lower bleeding risks and are currently undergoing preclinical and clinical trials. With safer therapies on the horizon, cardiovascular diseases may become a manageable disease, potentially reducing the health burden of these diseases.

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