

Platelet receptors and patient responses: The contributions of Professor Stan Heptinstall to platelet research.

Kenneth J. Clemetson, Haemostasis Research, Dept. of Haematology, Inselspital, University of Berne, CH-3010 Berne, Switzerland

## Abstract

Stan Heptinstall's contributions to platelet research covered organising meetings at the national and European level as well as starting and maintaining the journal 'Platelets'. The major part of his research addressed problems of inhibition of platelet receptors and the effects of this on patient health. In particular, the effects of P2Y<sub>12</sub> inhibitors on patients, with acute cardiovascular problems was a major focus. Other studies included the effects of feverfew (*Tanacetum parthenium*) extracts on platelets, of direct anti IIb/IIIa receptor ( $\alpha$ IIb $\beta$ 3) inhibitors and of prostanoids on platelet function. Recently, methods for assessing the effectiveness of platelet inhibition were investigated.

## Introduction

Stan has contributed to platelet research on a number of levels. He has been involved in a large amount of basic and clinical research on platelets and adjoining areas and these will be discussed below but his contributions to what may be called the sociology of platelet research are also large and need to be considered too because in the long term they may have had even greater effects.

We are now used to research being organised on a European and international level but when Stan began his research, apart from a few major areas such as CERN, astronomy and space research where it was difficult for individual countries to make meaningful contributions because of the scale required, most research was done essentially at a local, national level. This does not mean that there was no international collaboration in the "smaller" areas such as platelet research but this was often organised on a very *ad hoc* basis – but was no less effective for that. Stan saw that there were problems, particularly in respect of people living behind the – then – Iron Curtain, and because many of these faced severe travel restrictions. One of the main ways in which he and his colleagues tried to help was by encouraging Western researchers to participate in the Erfurt Conferences organised regularly by DDR colleagues and also attended by other Eastern European colleagues, even from as far away as Russia. After the lifting of travel restrictions in 1989 these meetings were well attended by researchers from across Europe and even further afield and continued for many years. More recently there has been a trend to consolidate platelet related meetings and to hold them in various sites across Europe, often together with national meetings.

Another major initiative that Stan took was, of course, as inaugural editor in the launching of the journal "Platelets" in 1990. Professor Dimitri Mikailidis had persuaded Churchill Livingstone to publish this new journal and Stan was proposed as editor. Stan discussed this with many of us before going ahead. He had several motivations: There was no journal devoted solely to platelet research, all the other journals had a broader basis either in thrombosis and haemostasis or in haematology and several around that time, perhaps lacking forceful enough editors, had lost support and influence. In addition, Stan wanted to provide a platform for

authors outside the mainstream and also in developing countries to help them reach a bigger audience and to encourage them in their research. As part of this he regularly published abstracts from platelet meetings such as those mentioned above. The first Erfurt Conference Abstracts to appear in Platelets were those for the 4<sup>th</sup> Meeting in 1992 [1]. For a number of years it was difficult to attract adequate numbers of papers of interest to readers (perhaps because papers in the platelet area did not expand as rapidly as the scientific literature in general) but the situation improved and today the journal is in the “happy” situation of receiving more reasonable quality papers than it has space to publish and must therefore reject quite a few. However, it has indeed managed to extend its authors to a wide range of countries previously poorly represented. There was a major scare at the end of 1995 when the then publisher decided to stop but Stan managed to find a new publisher and continue and since then the journal has gone from strength to strength. Thus, Stan has managed to achieve his major goal in leaving “Platelets” as a healthy journal meeting most of his objectives. The detailed history of Platelets is dealt with by Alan Nurden in the accompanying chapter.

Since 1985 Stan has been professor of thrombosis and haemostasis and is the main coordinator of the Clinical Sciences Homebase at the University of Nottingham, which contributes to the teaching of more than 50 third year medical students studying for a Bachelor of Medical Sciences degree. In his recent role as head of the Division of Cardiovascular Medicine, he was involved in employing, mentoring, and guiding staff working closely with their National Health Service colleagues at both Nottingham City Hospital and Nottingham University Hospital as well as with colleagues involved in basic research. He remains an active member of the International Society for Thrombosis and Haemostasis as well as the British Society for Haemostasis and Thrombosis, where he was an honorary secretary and also had a turn as president.

Turning to his research interests, after a few years working on bacterial cell wall components, the subject of his doctoral thesis, Stan settled down essentially to what would be his long term interest. Of course, this concerned platelets but in particular it concerned how platelet receptor inhibition could protect patients, people at high risk or even ‘normal’ individuals against thrombosis and other cardiovascular diseases without the major side effect of an increased tendency to bleeding. One of the first breakthroughs in this area had been the demonstration that aspirin protects patients against thrombosis, later shown to be via inhibition of platelet thromboxane synthesis. That this was possible awakened considerable interest in alternative methods of inhibiting platelet activation.

Following the demonstration that the bleeding disorder Glanzmann’s thrombasthenia was caused by a defect or deficiency in the major platelet integrin  $\alpha\text{IIb}\beta\text{3}$ , preventing platelet aggregation via fibrinogen, considerable effort was exerted to produce synthetic inhibitors to cause a similar but controllable blockage. This led to the development of peptide or antibody inhibitors of  $\alpha\text{IIb}\beta\text{3}$  and then small molecule inhibitors, used extensively for prevention of thrombosis in acute problems, particularly in surgery. The hunt was then on for small molecule inhibitors that could

be used for treatment of chronic disease.

Already in 1975 the thienopyridine anti-thrombotic drug ticlopidine was shown to be an efficient platelet aggregation inhibitor but following side effects was replaced by clopidogrel in 1987 and, in 2001, their target was shown to be the ADP receptor P2Y<sub>12</sub>. The question arose as to how well each category and combination of inhibitors worked in patients. More recently, a major question has been why part of the population does not respond well to either treatment and may need different dosing. This is often referred to as resistance. The newest drug in this thienopyridine class is prasugrel with clear dosage, efficacy and compliance advantages.

Stan's most cited paper (1994) is a large clinical study (cited 2241 times, as I write) dealing with thrombosis prevention in cohorts of patients and controls when treated with an anti-platelet regimen (aspirin) for longer periods (one to several years) and indicating that, at least in patients with cardiovascular problems, longer than conventional treatment did lead to increased protection [2]. However, there was little effect in controls (maybe the level of events was too low for statistically relevant results?). This was one of the first papers to indicate that extending anti-platelet therapy increased protection suggesting (often confirmed since) that vascular problems continue long after primary treatment for acute disease.

His second most cited paper (343) dealt with assessing the pharmacodynamics, pharmacokinetics, and safety of AZD6140, (later called ticagrelor) the first oral, reversible adenosine diphosphate (ADP) receptor antagonist to be used clinically [3]. Problems associated with clopidogrel and other thienopyridines include patient "resistance" i.e. not all patients receiving a standard dose showed adequate inhibition (ca. 80%) of the ADP receptor as well as the fact that the activate metabolite of clopidogrel reacts covalently with the P2Y<sub>12</sub> ADP receptor. This means that if surgery is urgently necessary, such as following an accident, clopidogrel treatment cannot be rapidly reversed and even if stopped it requires several days until enough fresh platelets are formed to reinstate full haemostasis. Thus, reversible (i.e. non-covalent) P2Y<sub>12</sub> inhibitors make sense, on the one hand to "top up" patients with inefficient inhibition by clopidogrel and similar treatment and on the other to treat patients where the risk of surgical intervention is high and where ceasing treatment leads rapidly to elimination of the inhibitor.

His third most cited paper (208) also falls in this area comparing effects and efficiency of AZD6140 with clopidogrel in patients with acute coronary syndrome [4]. Again, this refers to their relative efficiency in reducing a tendency to thrombosis and the problem of variation in response among patients. Stan also contributed to a long series of papers establishing the relative efficacy of P2Y<sub>12</sub> inhibitors and their role in dosing regimens for patients with or without aspirin as supplementary inhibitor. These have contributed in the long term to protecting patients from thrombosis and making surgery safer by reducing both bleeding and thrombosis risks.

Ticagrelor (AZD6140) is a cyclopentyl-triazolo-pyrimidine, a new chemical class of P2Y<sub>12</sub> antagonist that is now approved for use in a wide spectrum of acute coronary syndromes. Ticagrelor has a different binding site on P2Y<sub>12</sub> from ADP or cangrelor (an ADP analogue, making it an allosteric antagonist, with reversible blockage. The drug does not need hepatic activation, avoiding the problems with patients with

genetic variants of the enzyme CYP2C19 and has a longer in vivo half-life so that patients need only be dosed once a day giving better compliance.

Since some reversible P2Y<sub>12</sub> inhibitors e.g. cangrelor, compete for the same receptors as the covalent, irreversible ones (clopidogrel, prasogrel) it could be expected that if given first or even simultaneously they might prevent efficient irreversible inhibition and this was indeed demonstrated by Stan and his colleagues [5]. Although this might have been predicted it was still necessary to establish this experimentally because the relative binding constants of cangrelor and the activate metabolite of clopidogrel were not known.

Another interesting area of Stan's research has been the effects of feverfew (*Tanacetum parthenium*) extracts on platelets [6]. Feverfew is an anti-inflammatory medicinal herb with a long history of use in "natural" treatment of health problems including migraine. The main active principle is parthenolide, now also a promising candidate for anti-cancer treatment. Parthenolide is a sesquiterpene lactone that, like clopidogrel, is activated by P450 (CYP2C19). However, it is active against signalling pathways in cancer stem cells. In platelets it seems to act by inhibiting –SH groups (perhaps also disulphide isomerase) involved in signalling pathways. Stan also contributed to improved methods for extracting parthenolide from feverfew, an important step in preparing pure material for testing biological activity.

In the late 80s and early 90s there was much interest in the development of direct anti IIb/IIIa receptor ( $\alpha$ IIb $\beta$ 3) inhibitors to prevent thrombosis and several have been successfully applied clinically in acute situations. These are a cyclic peptide based on snake venom protein disintegrin sequence (integrilin, eptifibatide) and a humanised monoclonal antibody (abciximab) and more recently a small molecule inhibitor (tirofiban). However, attempts to develop small molecule antagonists that could be used for long term treatment of chronic conditions were unsuccessful in clinical trials mainly because they activated the  $\alpha$ IIb $\beta$ 3 so that fibrinogen could cause platelet aggregation and activation. Although it was clear that alternative inhibitors should be sought that do not activate  $\alpha$ IIb $\beta$ 3 or cause thrombocytopenia via neoepitope formation, the pharmaceutical companies have been reluctant to pursue this path. Stan and his colleagues did a number of studies with the available anti-IIb/IIIa inhibitors to explore some of their side effects on platelets. These included the investigation of platelet-leukocyte and platelet-monocyte conjugates because although platelet aggregation was inhibited, platelet activation and release of granules and expression of granule membrane proteins such as P-selectin and CD154 were only partially inhibited. Treatment of the conjugates with EDTA dissociated the platelets from the nucleated cells but did not dissociate P-selectin or CD154 from the platelets. The conclusion reached was that despite inhibiting aggregation, conjugate formation was enhanced favouring inflammation and thrombosis and providing an explanation for at least some of the (originally at least, unexpected) negative clinical effects of this class of IIb/IIIa inhibitor [7]. More recently, there has been renewed interest in this problem in the hope of developing anti- $\alpha$ IIb $\beta$ 3 molecules for injection as emergency treatment for myocardial infarction to help to disrupt a blocking thrombus.

I have dealt here with some of the main areas of Stan's research. However, over the years he dealt with a great many other aspects of platelet activation and inhibition via

a wide range of receptors and/or signalling pathways. Although none of these has been adapted clinically for a variety of reasons they were (are) nevertheless of considerable interest in understanding how platelets work and how their activity is regulated *in vivo*, which is vital for long term development of better platelet activation inhibitors. With the development and clinical acceptance of prasugrel and ticagrelor it might be argued that optimal inhibitors are already here and certainly any new inhibitors will have to show considerable improvements in properties to replace or complement these. Even so there may still be scope for improvement, particularly in respect of stroke where prevention and treatment still have a long way to go and where progress has been somewhat inhibited by the lack of adequate animal models as well as considerable differences in physiology. In addition, in the future it may be necessary to have inhibitors that affect some aspects of platelet function but not others. Hence, research into the effects of other receptor pathways is still of considerable interest even if they do not find instant application.

In the circulation, platelet reactivity is normally regulated by an endothelial membrane apyrase (CD39) that destroys ADP released by platelets or erythrocytes and thus prevents platelet activation. However, under pathological conditions this may not be adequate which is why the P2Y<sub>12</sub> receptor inhibitors are effective. Platelets are also kept pacified by prostaglandin and NO release from endothelial cells. Platelet activation *ex vivo* where they are no longer exposed to these factors may therefore not completely reflect the *in vivo* situation. Stan and his colleagues were interested in defining how platelets were affected by prostaglandins and which receptors are involved. In 2011 they demonstrated that platelet responses to PGE<sub>1</sub> and PGE<sub>2</sub> are mediated by different prostanoid receptors and in 2012 showed that like PGE<sub>2</sub>, PGE<sub>3</sub> interacts with EP3 and EP4 receptors, but not the IP receptor, which is responsible for mediating the inhibitory effects of PGE<sub>1</sub> [8]. Therefore, the overall effect of PGE<sub>3</sub> on platelet function, like those of PGE<sub>1</sub> and PGE<sub>2</sub>, reflect a balance between effects at both activating and inhibitory receptors. The mechanism of PGE<sub>3</sub> is particularly interesting because it is derived from omega-3 fatty acids, which in the diet are generally shown to have positive effects on the development of cardiovascular diseases. The study of platelet prostanoid receptors is relevant to the development of new anti-inflammatory drugs because some COX-2 specific antagonists appear to affect synthesis of prostaglandins controlling platelet activity which was unexpected.

In the years leading up to his retirement Stan was also interested in improved methodology for testing *ex vivo* levels of platelet activation and how these were affected by platelet inhibition. A typical approach is the measurement of VASP phosphorylation, which is altered, particularly via ADP receptor activation [9]. Thus, the levels of VASP phosphorylation can be used to estimate the degree to which the P2Y<sub>12</sub> receptor has been inhibited and therefore how much the patient is protected. This can also be followed by looking at P-selectin expression rates compared to controls [10].

Of course, "No man is an island", and Stan's research would not have been possible without the large number of colleagues who participated in various capacities, clinical, postdocs, graduate students and technicians. Figure 1 shows one of the last of these groups from 2010.

Thus, in summary, Stan and his colleagues have contributed largely to an improved

knowledge of platelet receptors and how these regulate platelet activity. They have tested a wide range of different classes of inhibitors and they have studied some of the most promising of these in clinical trials. Thus, they have helped to provide data on the effectiveness of these inhibitors that was invaluable for determining their clinical usefulness.

In this relatively short overview there is not enough space to cite all of Stan's publications and the reader must therefore refer to PubMed or similar to see all of these. Those references listed below include particularly relevant or representative works.

The author declares that he has no conflicts of interest.

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### Legend to Figure

Figure 1. Stan Heptinstall's team in 2010. Postdoctoral researchers Sue Fox, PhD, David Iyu, PhD, and Natalie Dovlatova, PhD, and technicians Jane May, Jackie Glenn, Ann White, and Andrew Johnson. Other external collaborators are not shown.



Figure 1